IAFWA Research Update

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IAFWA Research Update

Chloramine-T TAS

Toxicity Assessment

- Standard Treatment Regimen
  - 60-min exposures of 20 mg/L administered once daily on four consecutive days
- Assess acute toxicity
- Gross necropsies
- Feeding behavior
- Effects of
  - temperature: WAE - 15, 20, or 25°C; CCF - 22, 27, 32°C
  - exposure duration: 60 or 180 min (WAE and CCF)
  - life stage: fry vs. fingerling (WAE and CCF)
  - alkalinity and hardness: walleye only

Methods

- Chloramine-T - obtained from Akzo
- Concentrations - 0, 20, 60, 100, or 200 mg/L
- Coolwater fish tested (20°C)
  - walleye, northern pike, and lake sturgeon
- Warmwater fish tested (25°C)
  - channel catfish, largemouth bass, hybrid striped bass
- 15 L glass aquaria or 1 L glass aquaria

Walleye fry during
Cl-T exposure.

Necropsy of walleye fry
after Cl-T exposure.

Results: Gross necropsy and feeding behavior

- Pale gills in dead fish following treatment
- Pale translucent livers in northern pike that died following 200 mg/L treatment
- Feeding of walleye and channel catfish reduced by 100 and 200 mg/L treatment

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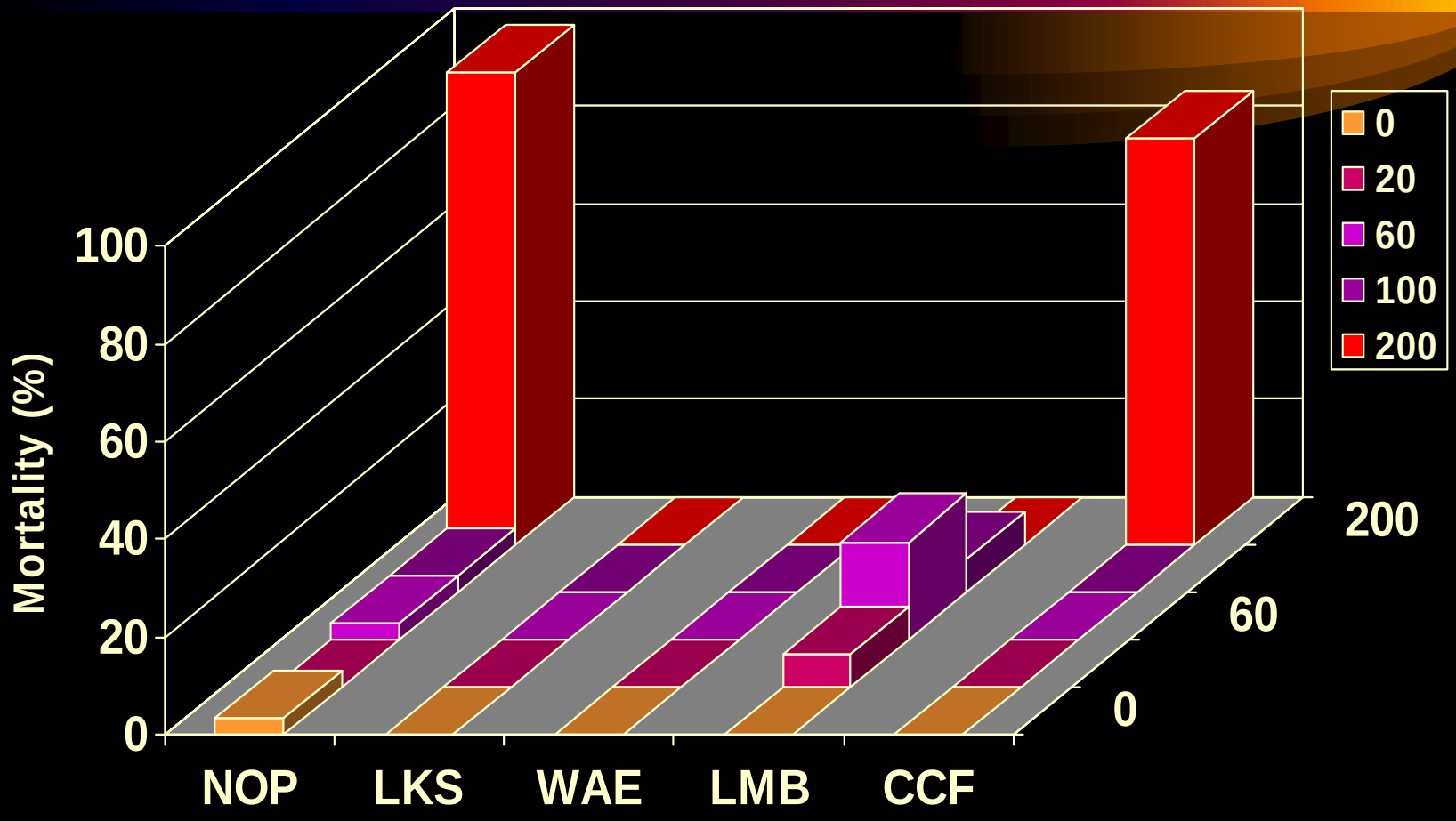
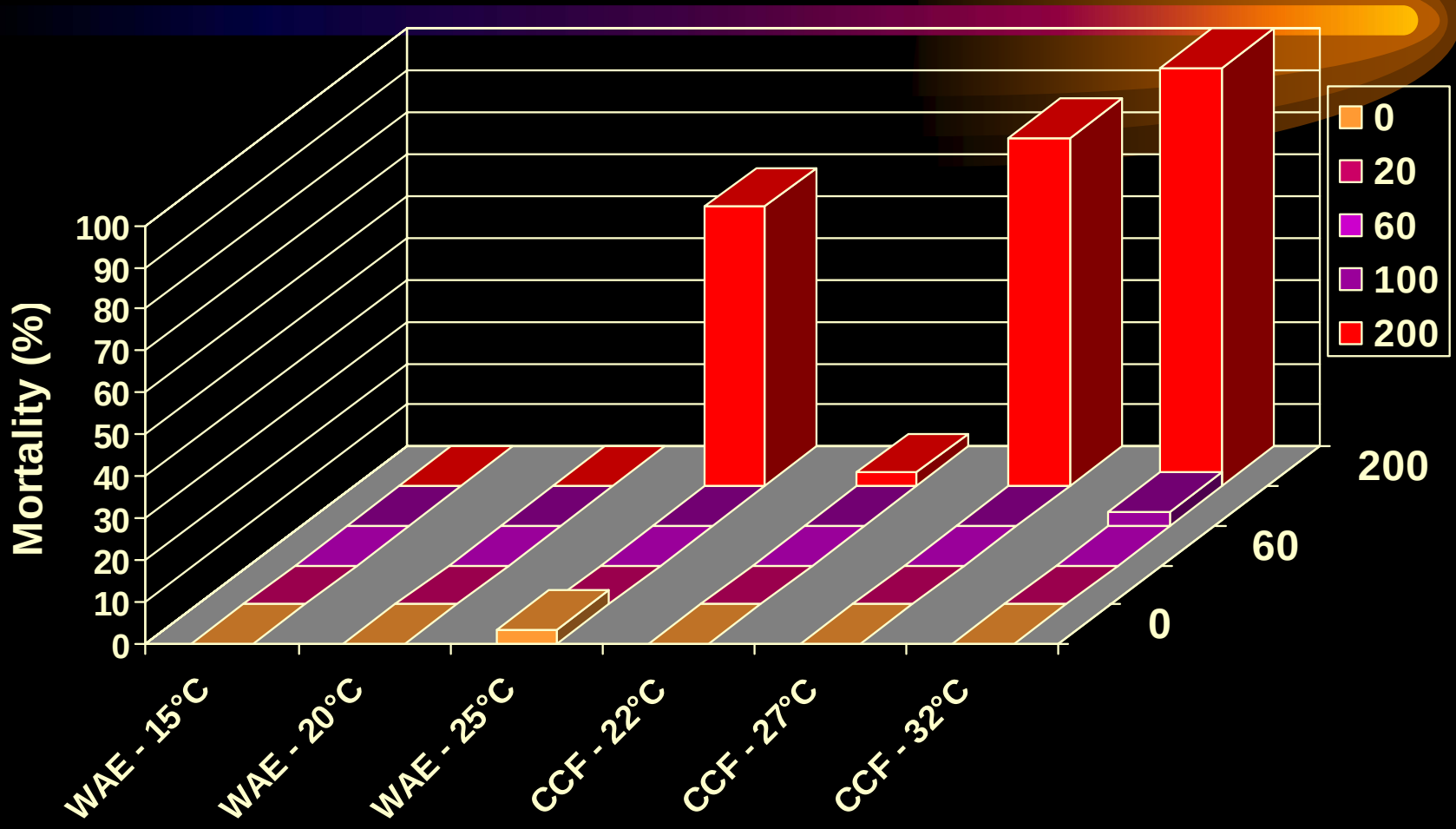
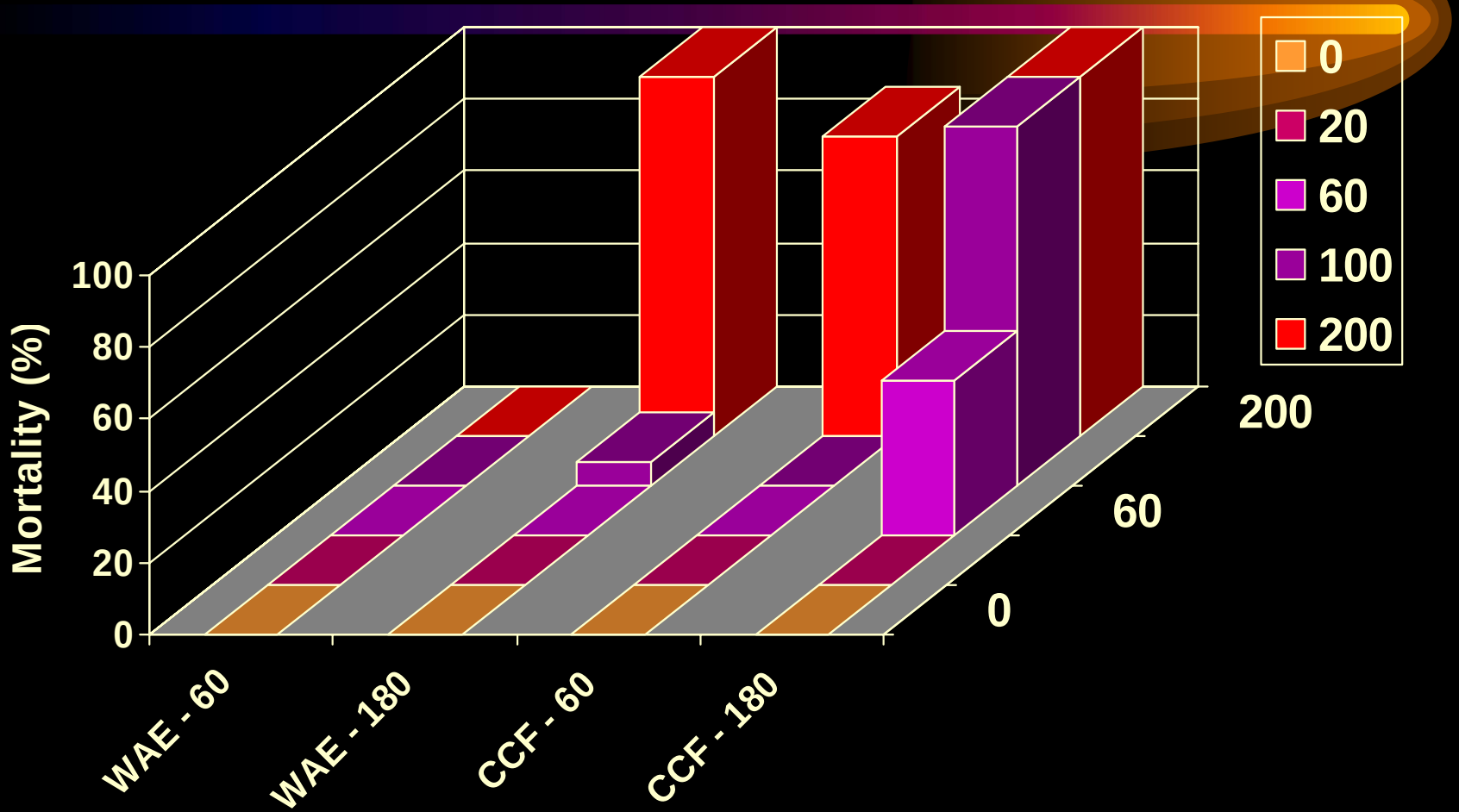
Cumulative percent mortality of fry exposed to Cl-T



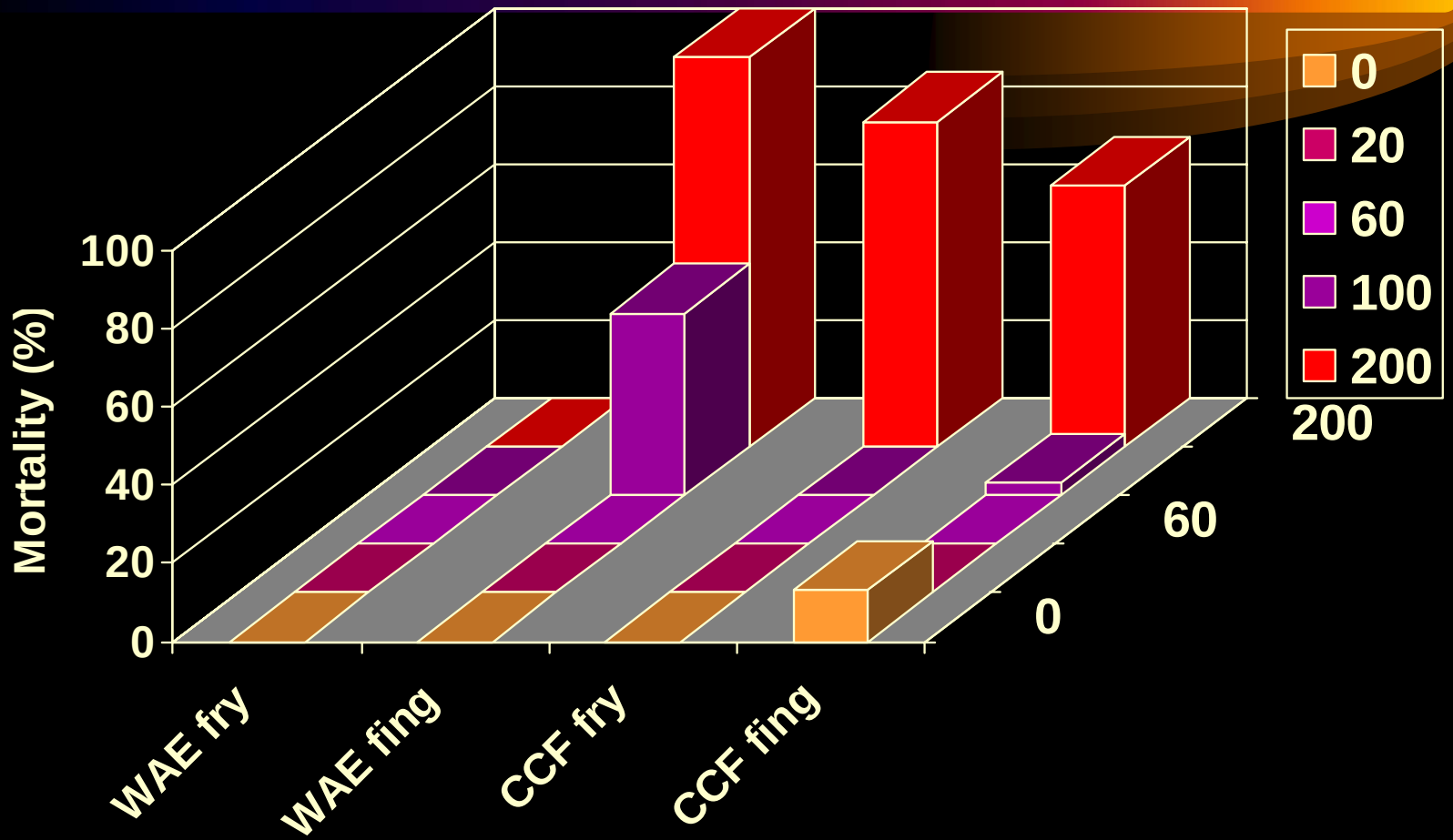
Effect of temperature on the toxicity of Cl-T to fry



Effect of exposure duration on the toxicity of Cl-T to fry



Effect of life stage on the toxicity of Cl-T



Studies in progress

- Evaluate the effects of soft water on the toxicity of chloramine-T to walleye fingerlings
- Prepare histological screening samples and show recovery after exaggerated treatment to walleye and channel catfish fingerlings

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Hydrogen peroxide TAS

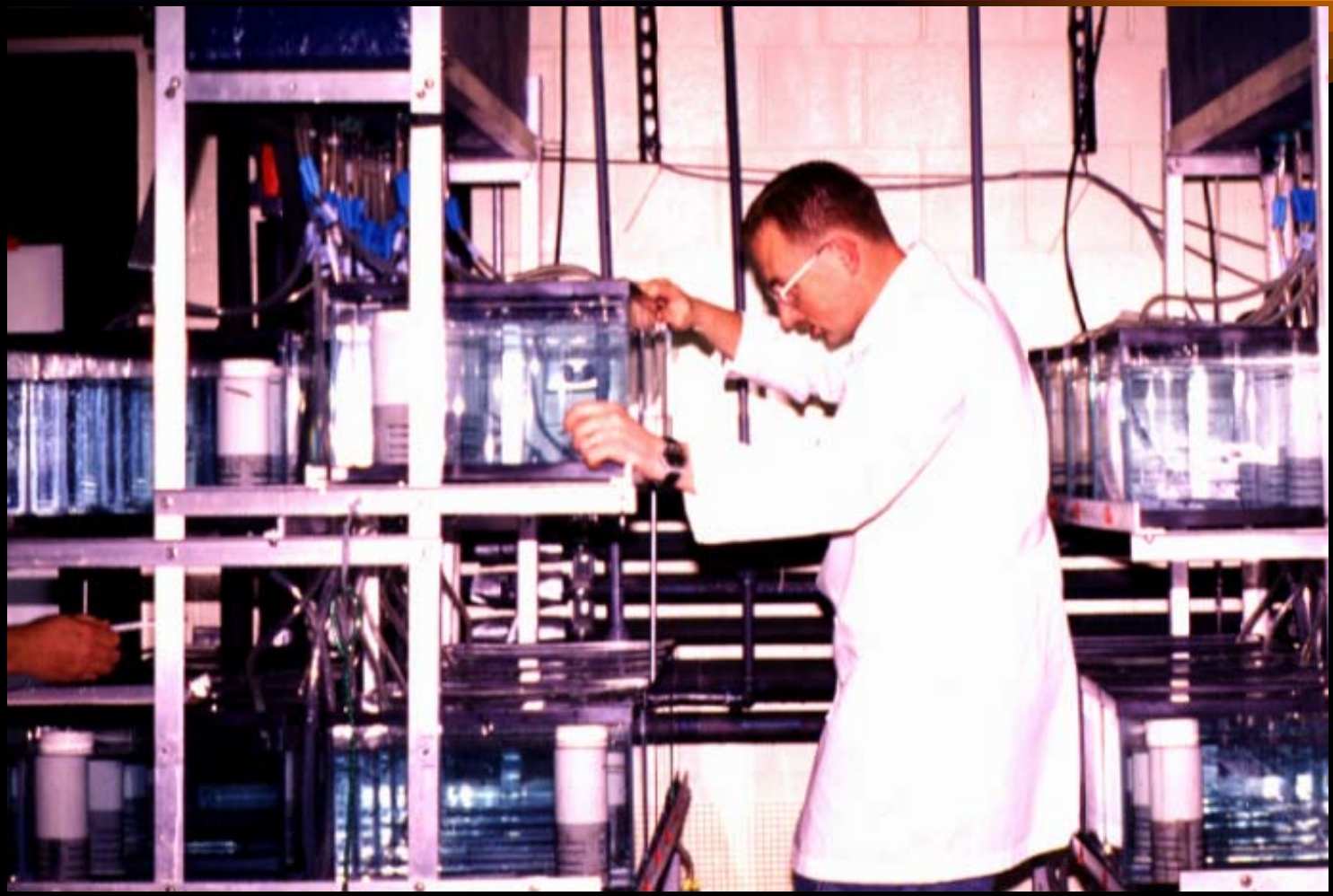
Objectives

- Determine the species most sensitive to hydrogen peroxide.
- Determine if life stages are similarly sensitive to hydrogen peroxide.
- Determine histological effects of hydrogen peroxide treatment to fish gills.

Methods and Materials

- Test Chemical
  - Hydrogen peroxide - 35% Food Grade, Du Pont Chemical Co
- Three exposures administered every-other-day for 60 or 180 min.
- High test concentration determined after range-finding exposures.

Exposure Systems



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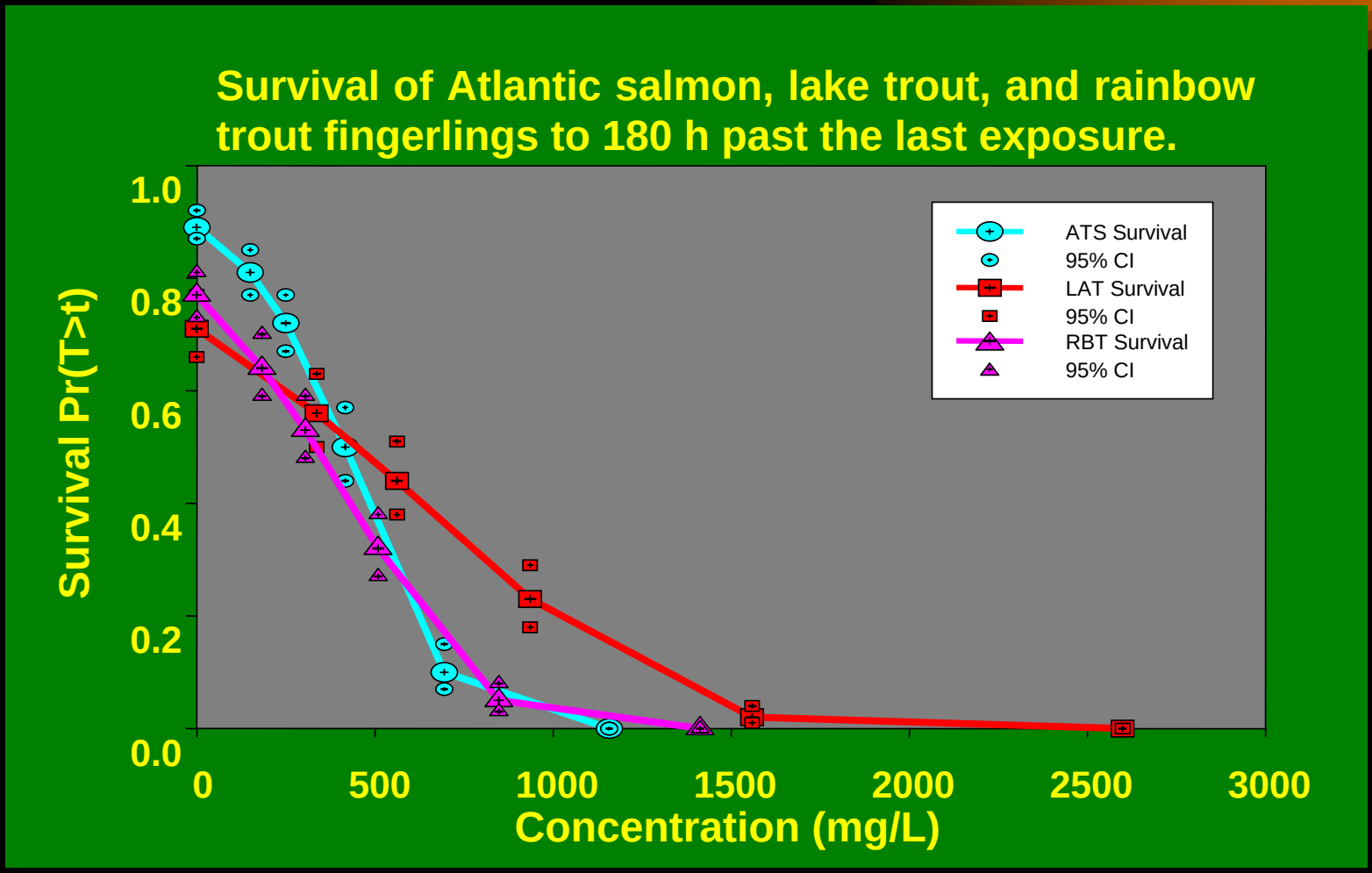
Methods

Species and Life stages Tested

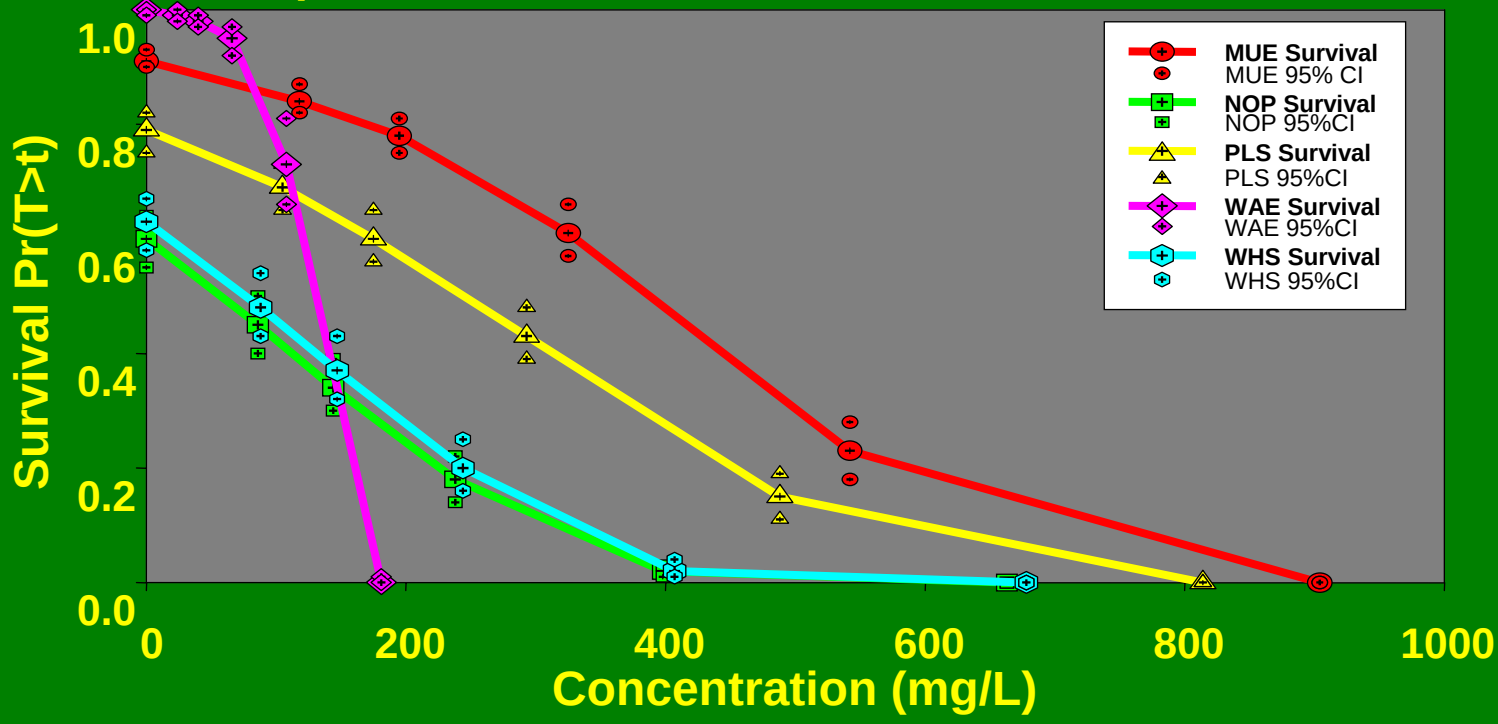
- Coldwater species - 12°C
  - Atlantic salmon and lake trout fingerling, rainbow trout fry and fingerling
- Coolwater species -17°C; fry and fingerling
  - muskellunge, northern pike, pallid sturgeon, walleye, white sucker
- Warmwater species - 22°C; fry and fingerling
  - bluegill, channel catfish, fathead minnow, largemouth bass, and yellow perch

Coldwater fingerling survival



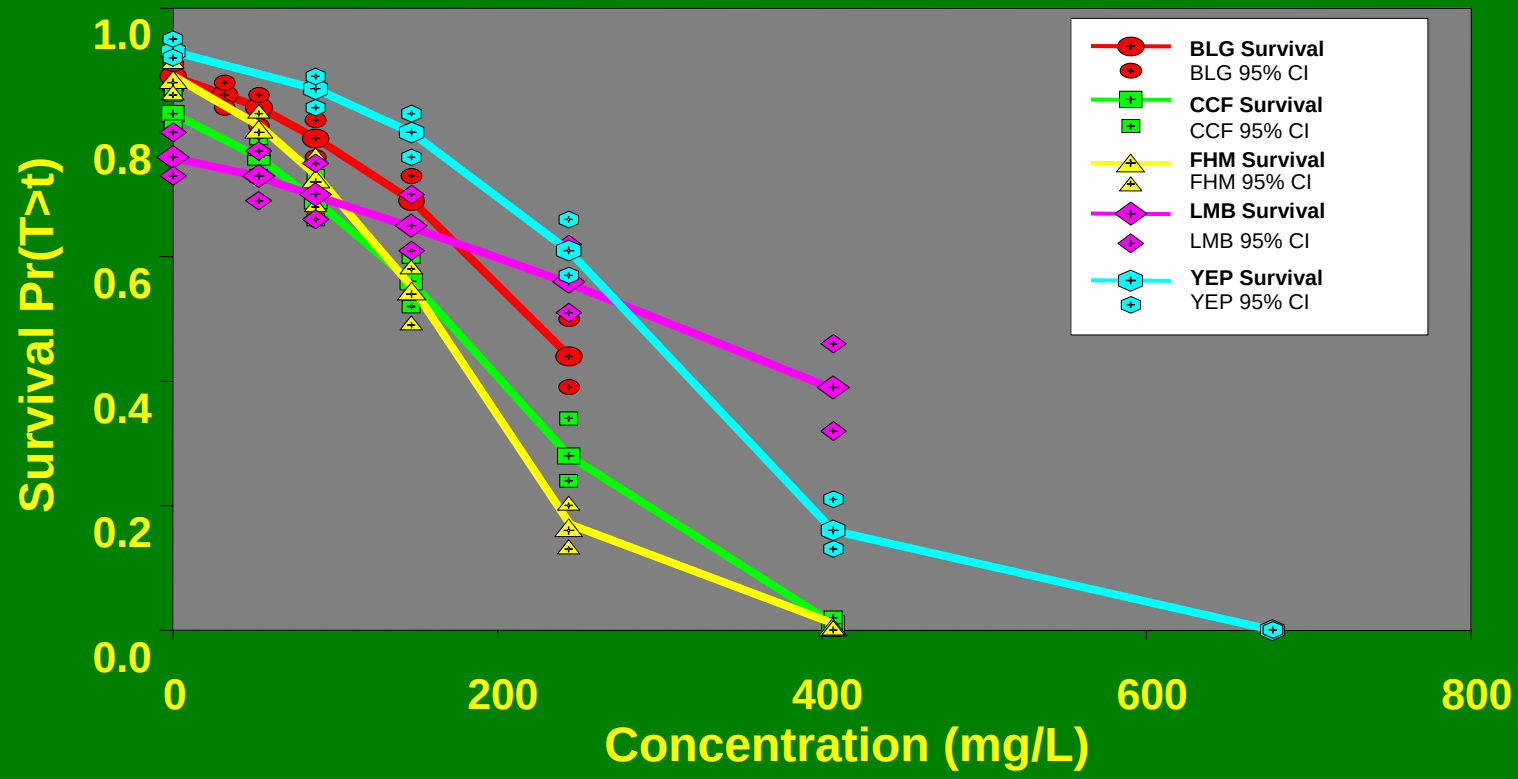
Coolwater fish survival

Survival of muskellunge, northern pike, pallid sturgeon, walleye, and white sucker fingerlings to 180 h past the last exposure.



Warmwater fish survival

Survival of bluegill, channel catfish, fathead minnow, largemouth bass, and yellow perch fingerlings to 180 h past the last exposure.



Toxicity Summary

- As a group, coldwater species were the least sensitive of the three groups tested.
- Coolwater and warmwater species were more sensitive to hydrogen peroxide exposure than coldwater species.

- Treatment suggestions by species - ≤ 60 min with > 90% survival

COLDWATER - 12°C

- **RBT fry - ≤170 mg/L**
- **ATS, LAT, and RBT fingerlings - ≤226 mg/L**

COOLWATER - 17°C

- **MUE fry/fingerlings - ≤113 mg/L**
- **NOP fry/fingerlings - ≤113 mg/L / ≤57 mg/L**
- **PLS fry/fingerlings - ?? / ≤113 mg/L**
- **WAE fry/fingerlings - ≤85 mg/L**
- **WHS fry/fingerlings ≤57 mg/L / ≤85 mg/L**

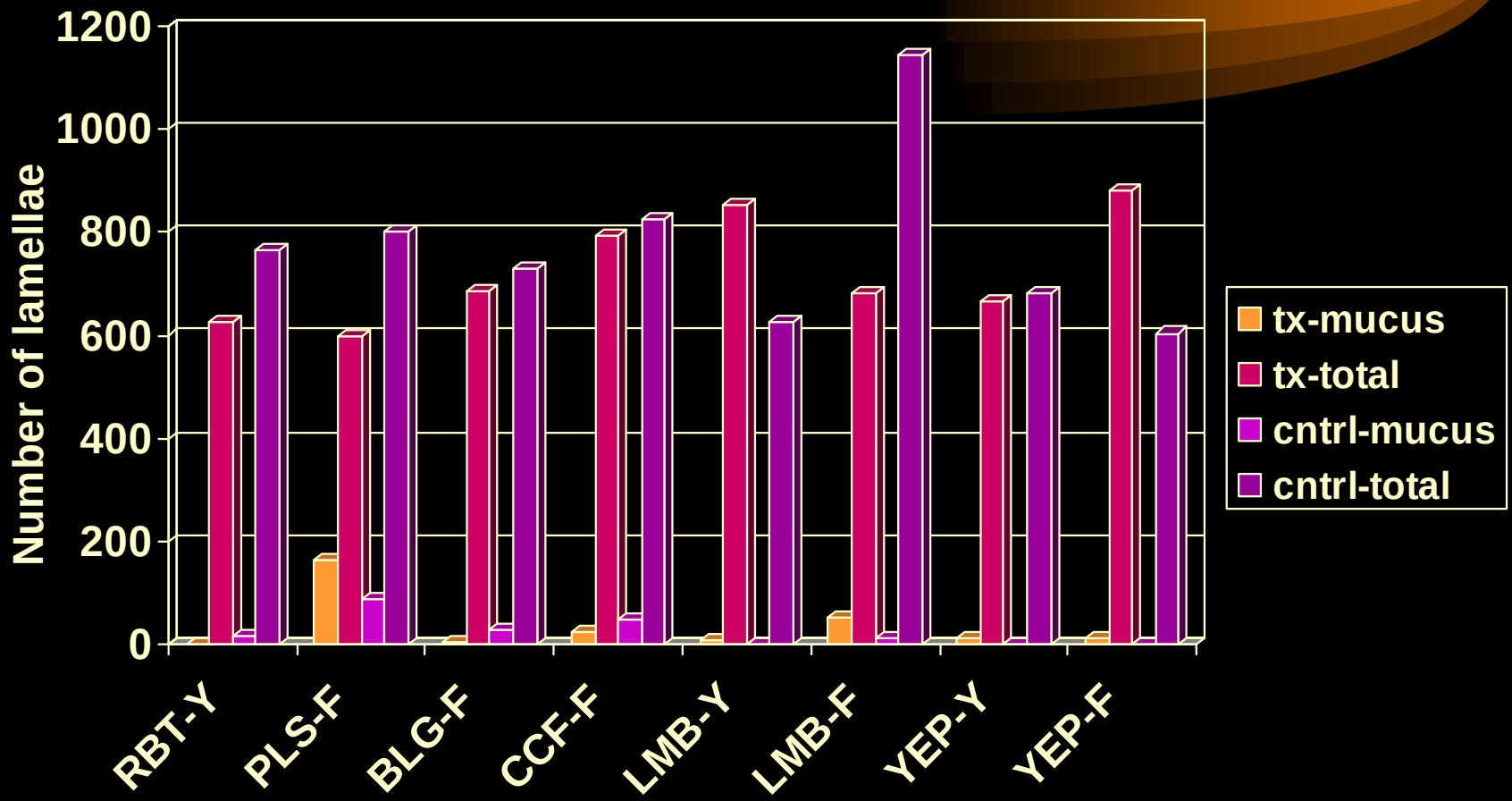
WARMWATER - 22°C

- **BLG fry/fingerlings - ≤85 mg/L**
- **CCF fry/fingerlings - ≤85 mg/L**
- **FHM fry/fingerlings - ≤57 mg/L / ≤85 mg/L**
- **LMB fry/fingerlings - ≤203 mg/L / ≤147 mg/L**
- **YEP fry/fingerlings - ≤57 mg/L / ≤85 mg/L**

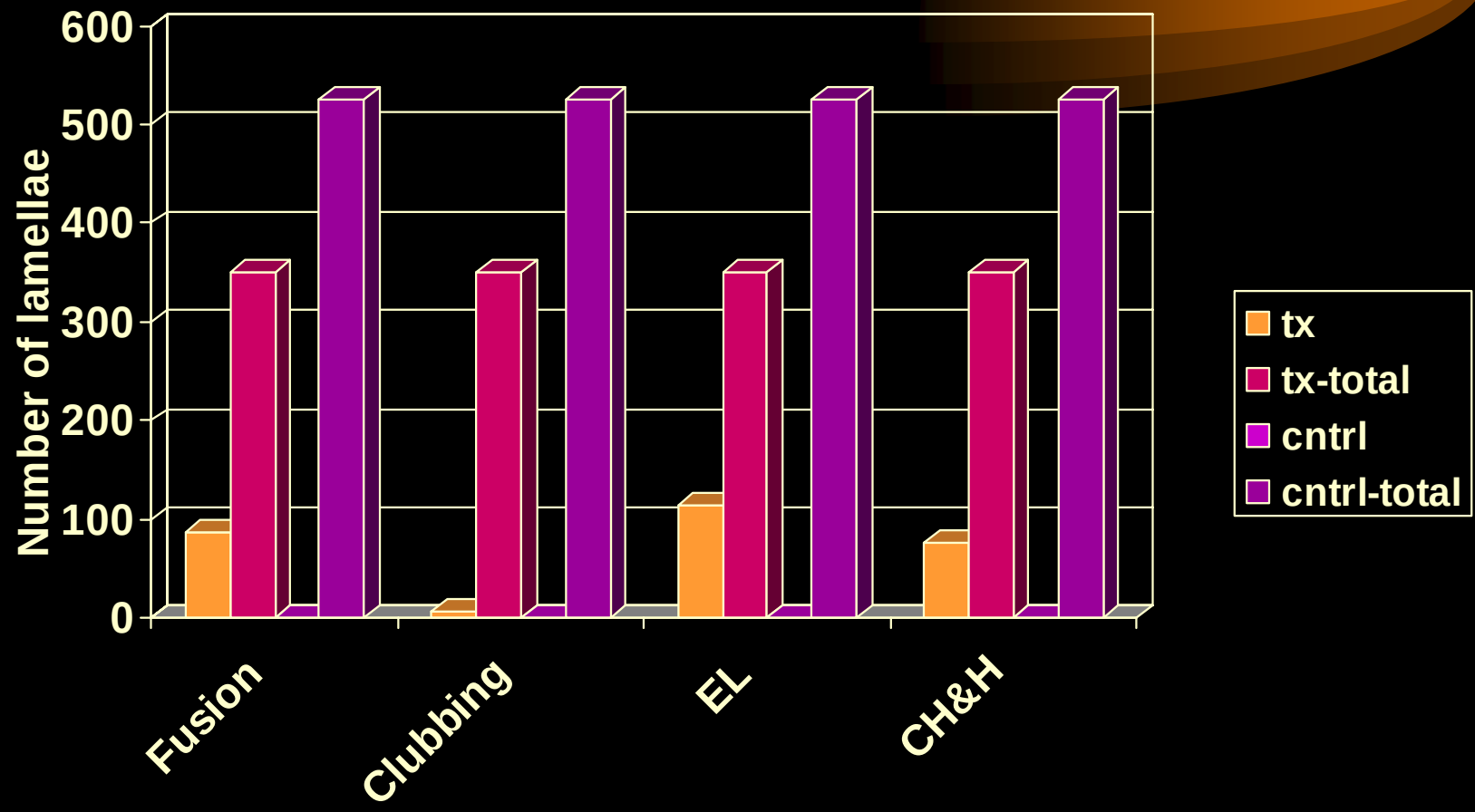
Histopathology

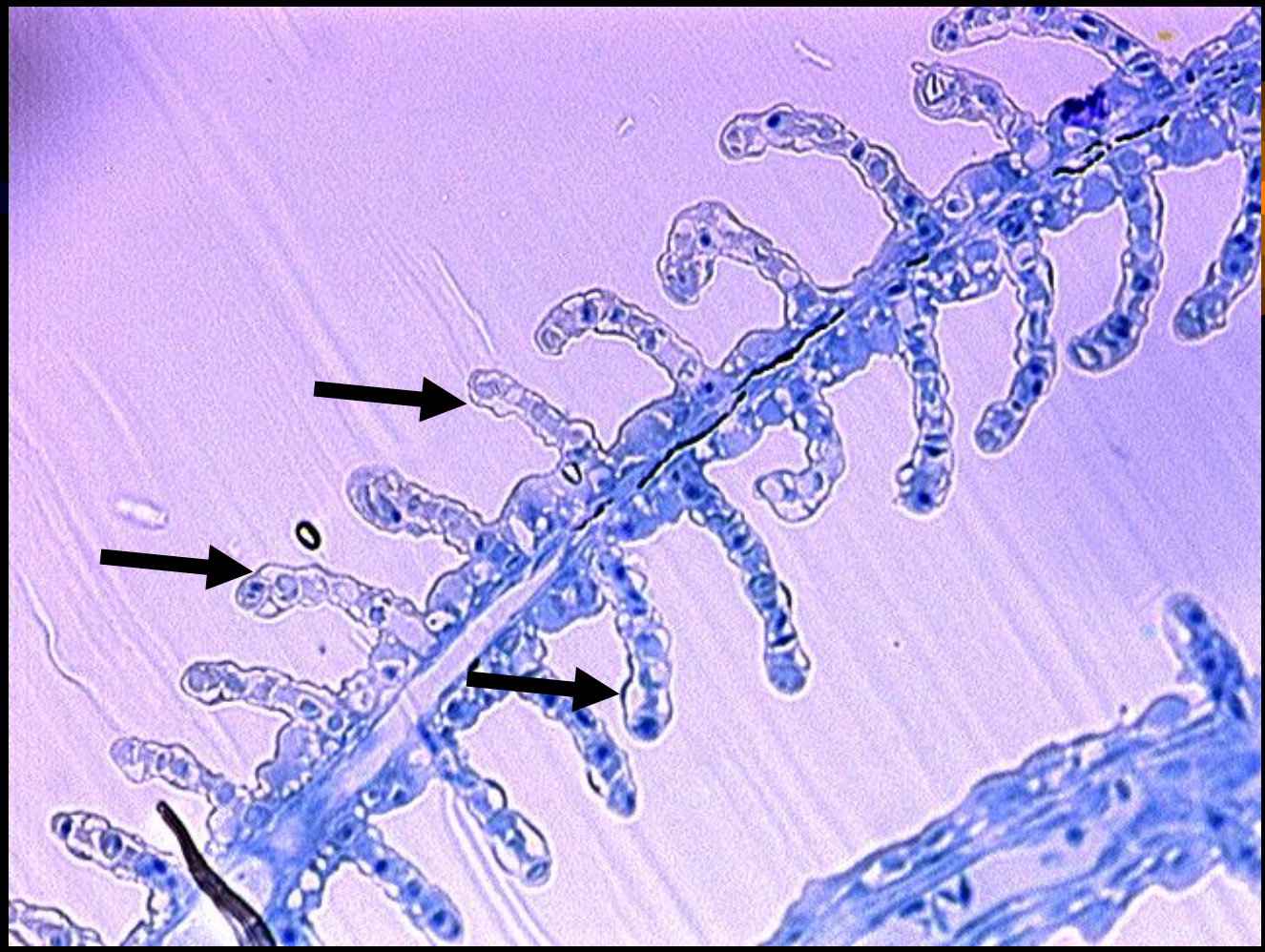
- Minor changes in most species
- Some pathologies (change in mucus or chloride cell number) may be a transient response to treatment
- Hydrogen peroxide treatment did induce
  - epithelial lifting in northern pike fingerlings
  - lamellar fusion in pallid sturgeon fry
  - ?? reduced respiratory capacity ??

Hyperplastic mucus cells



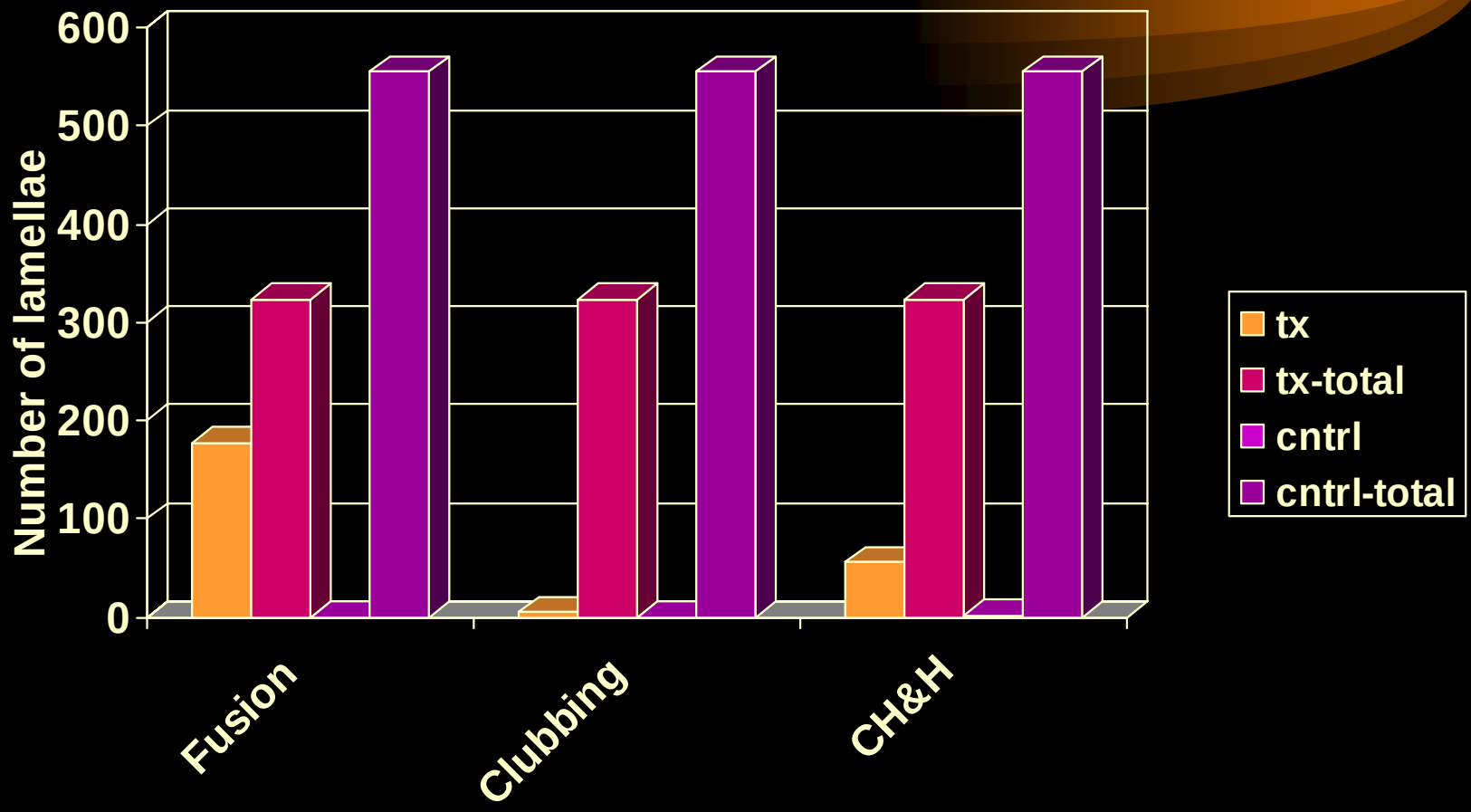
Northern pike fry

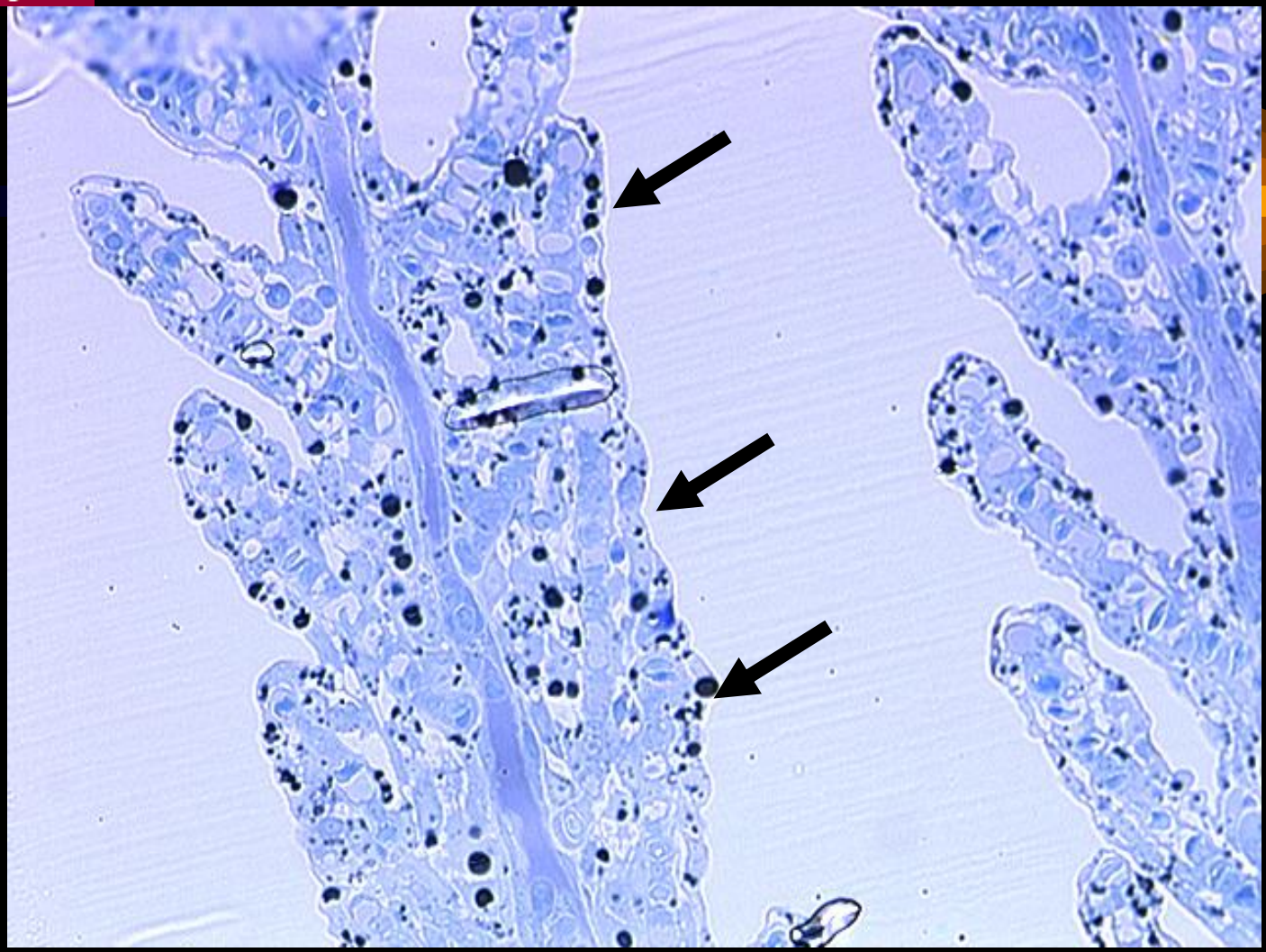




Epithelial lifting (arrows) in northern pike fry treated at 111 mg/L. (200X)

Pallid sturgeon fry





Severe lamellar fusion (arrows) in a pallid sturgeon fry treated at 215 mg/L. (200X)

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Hydrogen peroxide INAD (Perox-Aid™)

INAD #10-023

- Study CAP-00-FUNGUS
  - submitted to CVM 03 March 2000
  - thirteen participating facilities
- Study CAP-00-PARASITES
  - submitted to CVM 18 April 2000
  - eight participating facilities
- Study CAP-00-BACTERIA
  - submitted to CVM 18 April 2000
  - seven participating facilities

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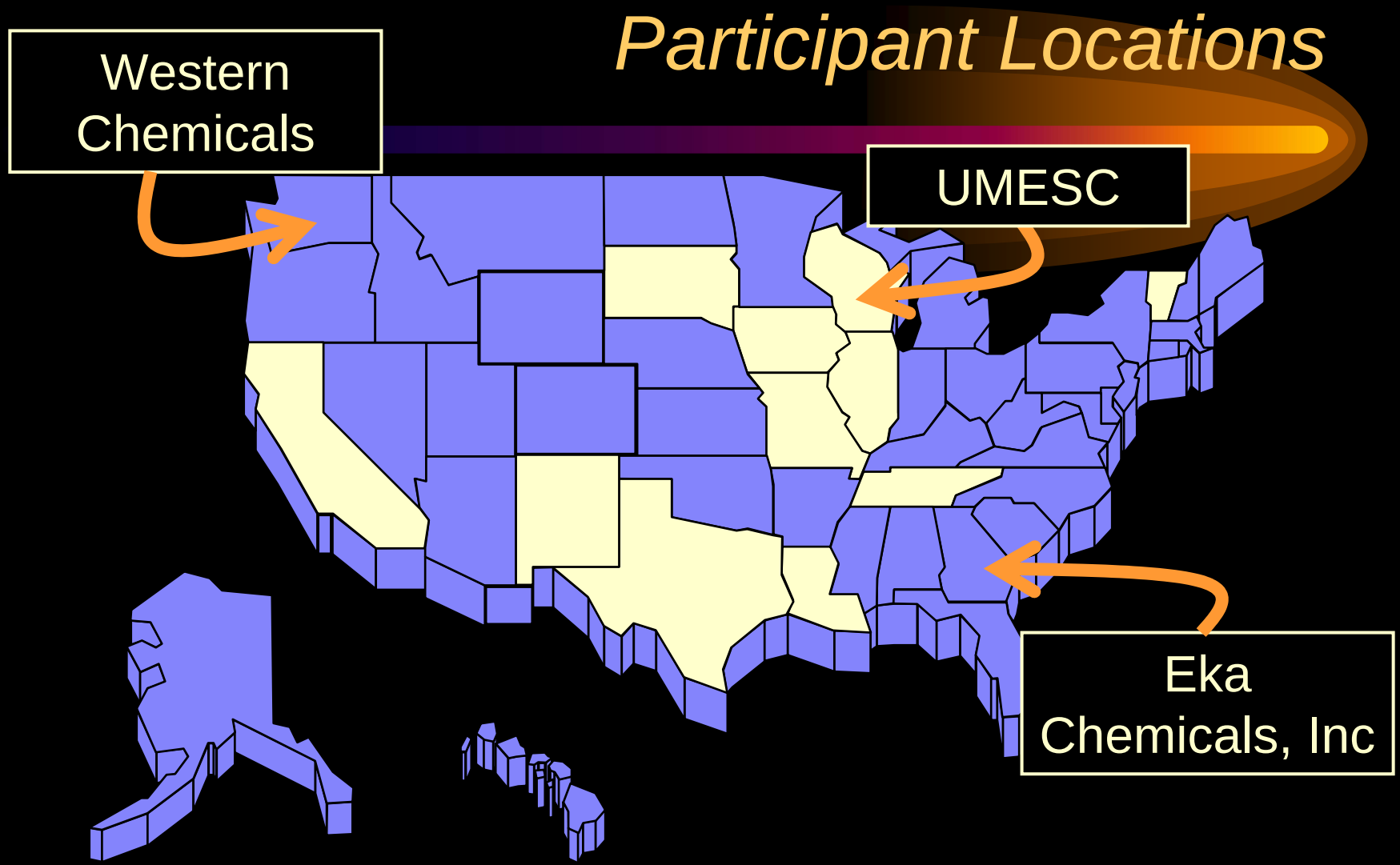
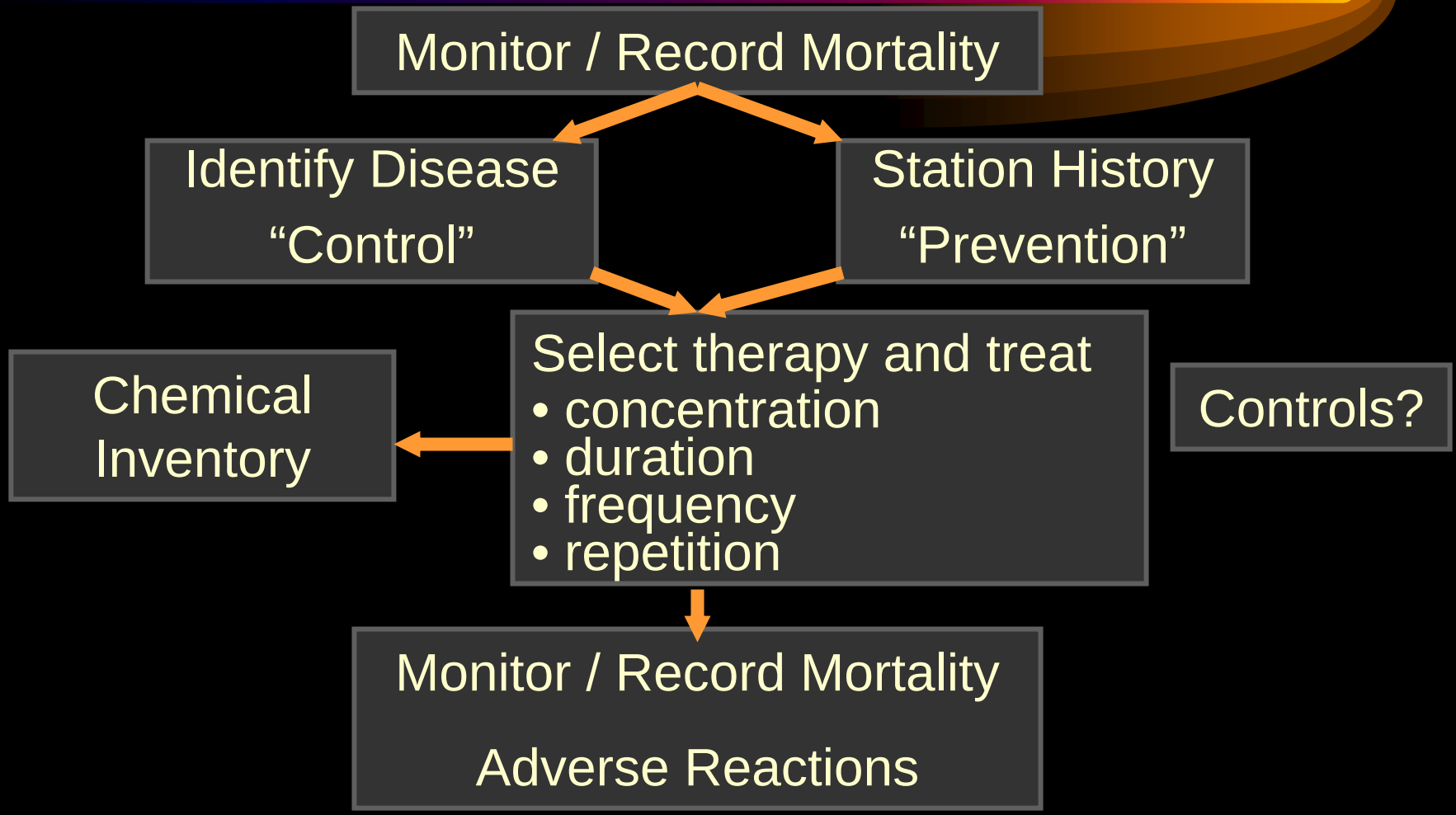
INAD #10-023
Participant Locations



Protocol Methods



Hydrogen peroxide therapy - Fish

- Duration: 30 or 60 min
- Concentration
  - 50, 75, or 100 mg/L for 60 min
  - 100 or 150 mg/L for 30 min
- Repetition/Interval: once daily on consecutive or alternate days for up to 10 treatments.

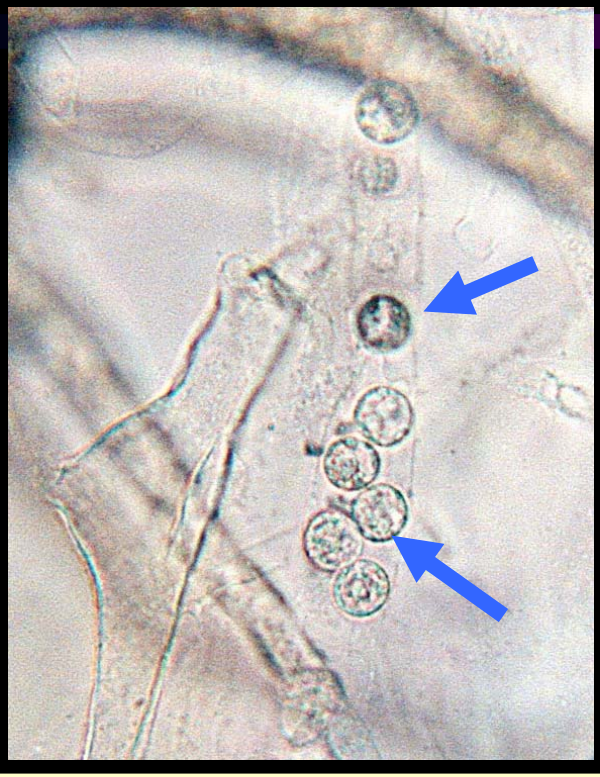
Hydrogen peroxide therapy - Eggs

- Duration: 15 min
- Concentration: 500 or 750 mg/L
  - minimum incubator concentration is 500 mg/L for 15 min
- Repetition/Interval: Once daily on consecutive or alternate days through hatch

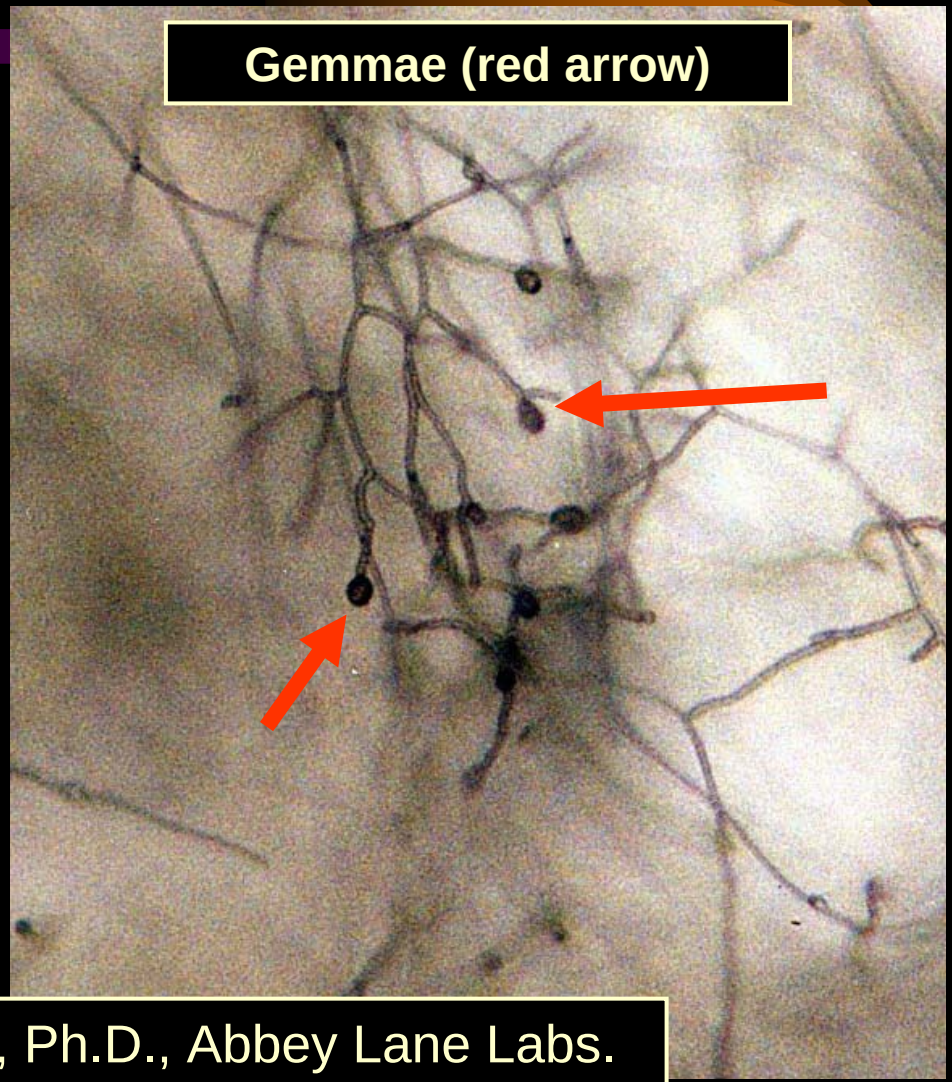
Egg Efficacy Trials

- Seven egg efficacy trials conducted
  - Blind Pony SFH, Jerry Hamilton, paddlefish
  - Blue Dog SFH, Clark Moen, walleye
  - Dundee SFH, Dennis Smith, smallmouth bass
  - Gavins Point NFH, Mark Drobish, walleye
  - Max McGraw, Tom Harder, walleye
  - Rathbun SFH, Alan Moore, channel catfish
  - UMESC, Lynn Lee, walleye
- Fungus identified in three trials

Saprolegnia parasitica



Zoospores in zoosporangium (blue arrow)



Gemmae (red arrow)

Photo credit: Steve Carpenter, Ph.D., Abbey Lane Labs.

Blind Pony SFH - paddlefish

- Temperature 15 - 16°C
- Four trials - one or two females per trial
  - 500, 750, or 1000 mg/L
  - % hatch ranged from 14 - 56% (of fertile eggs)
  - 2 - 4 consecutive treatments; fungus in all treated jars
  - noted cessation of egg development after hydrogen peroxide treatment
  - withheld treatments until 2 d post fertilization resulted in basically no hatch
  - compared rolling w/o treatment to hydrogen peroxide treatment w/o rolling
    - rolled eggs - 60% hatch treated eggs 24% hatch

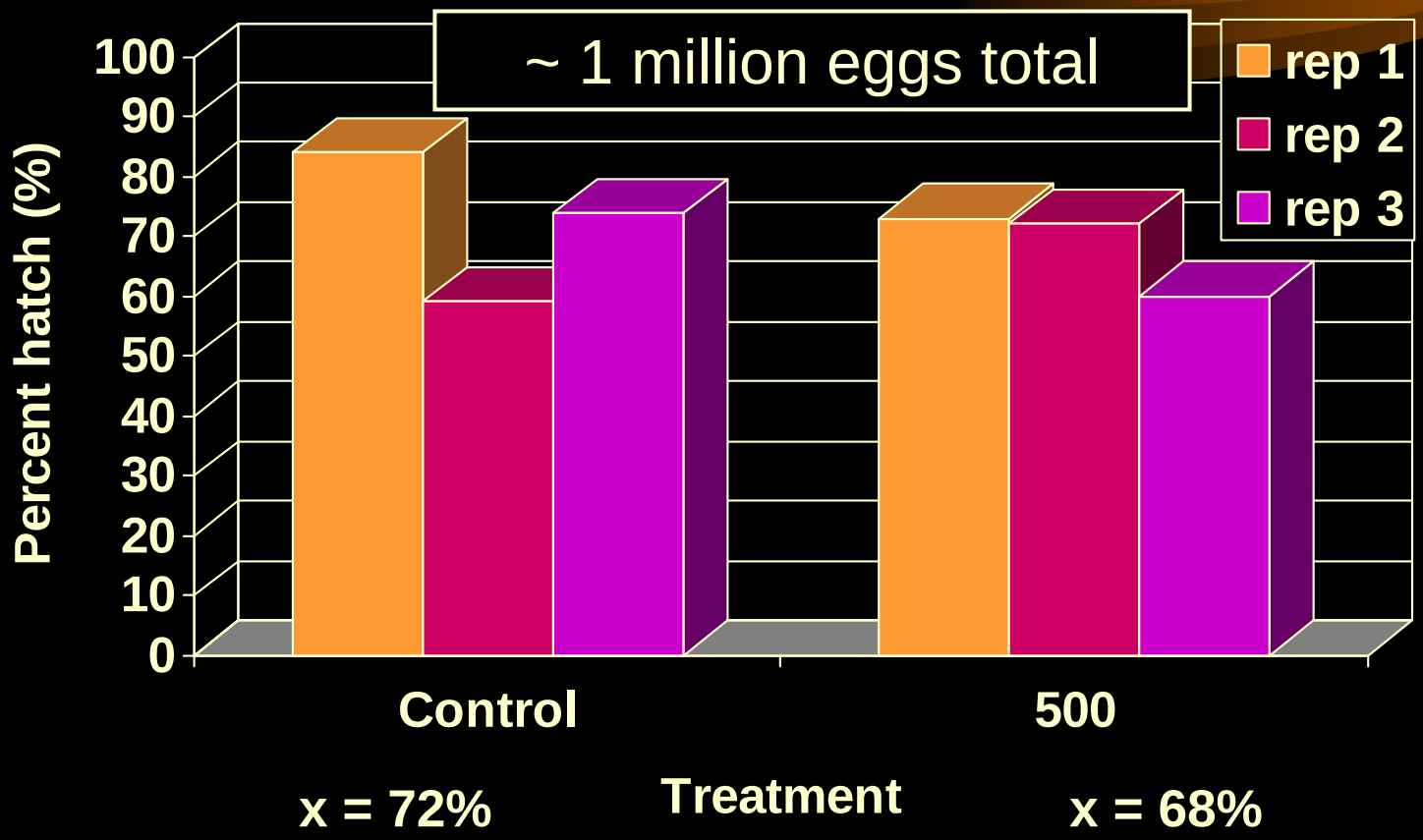
Blind Pony - paddlefish Saprolegnia ferax

Oogonium with oospores showing cell wall pitting.

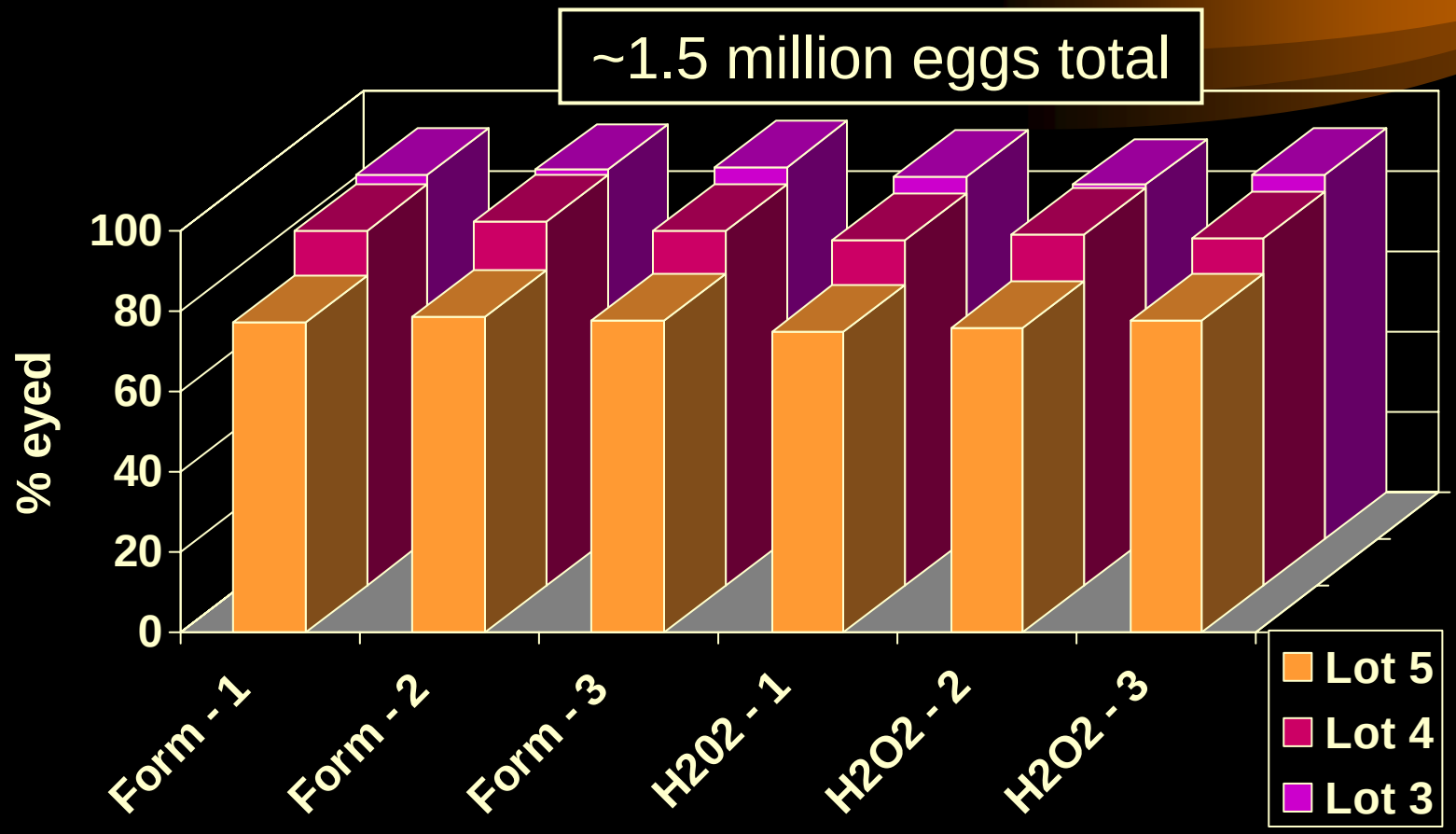
Oogonia and gemmae from sterile house fly culture.

Photo credit: Steve Carpenter, Ph.D., Abbey Lane Labs.

Max McGraw - walleye

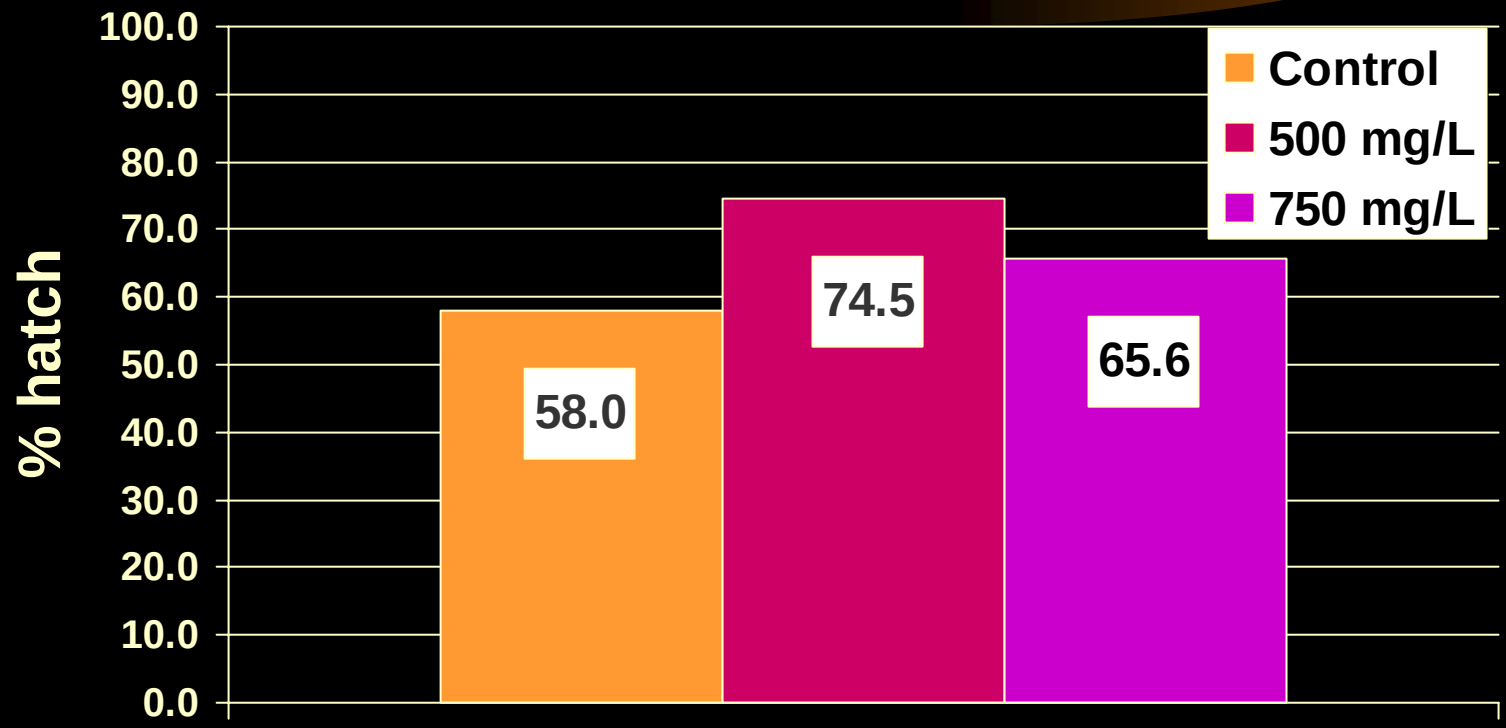


Blue Dog SFH - walleye



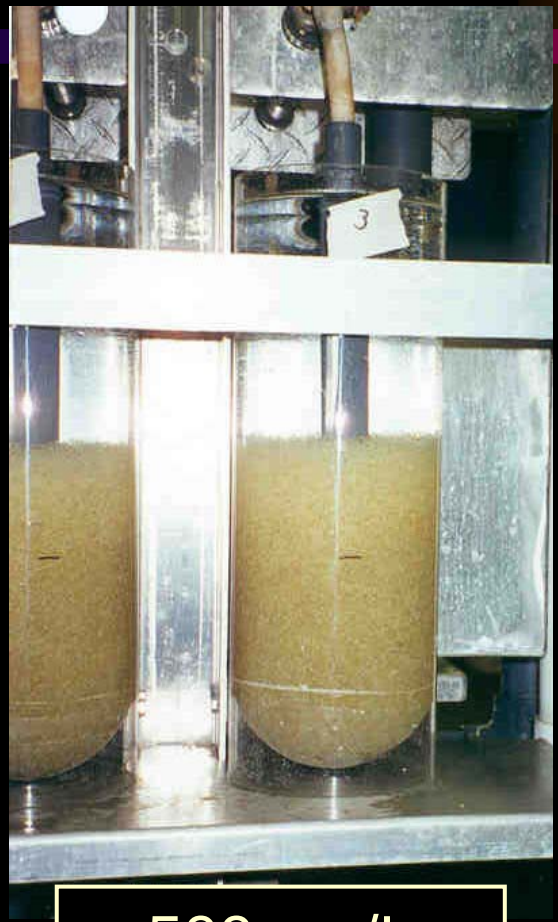
Gavins Point NFH - walleye

~3.1 million eggs total



Gavins Point NFH - walleye

Control



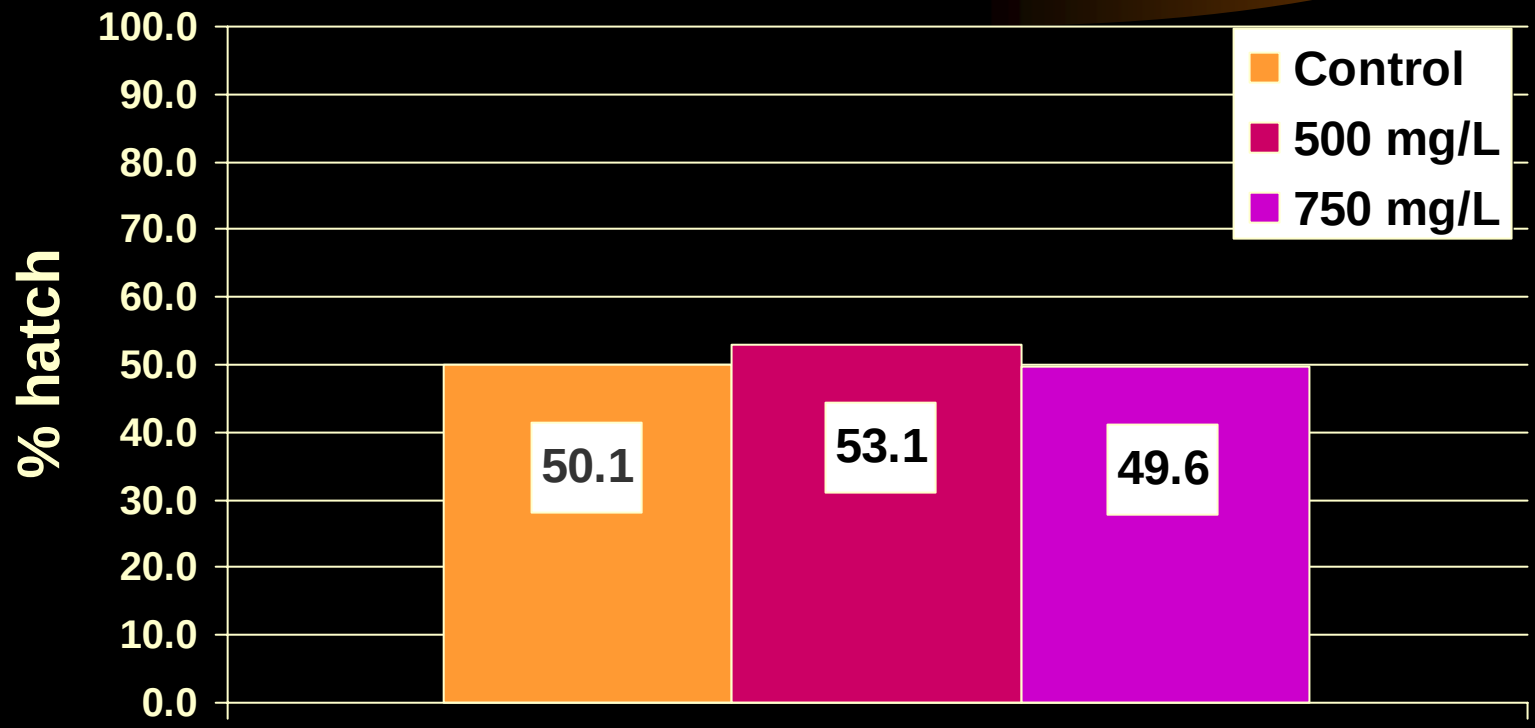
500 mg/L

750 mg/L

Photo credit: Mark Drobish, Gavins Point NFH

UMESC - walleye

~2.7 million eggs total



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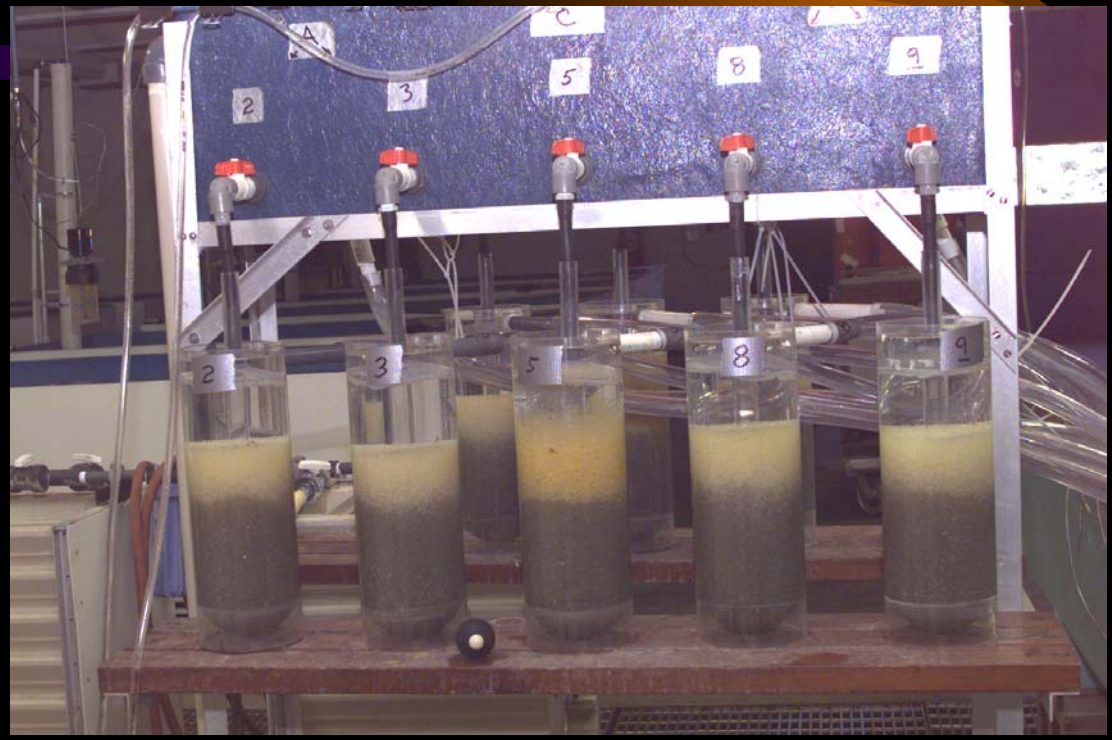
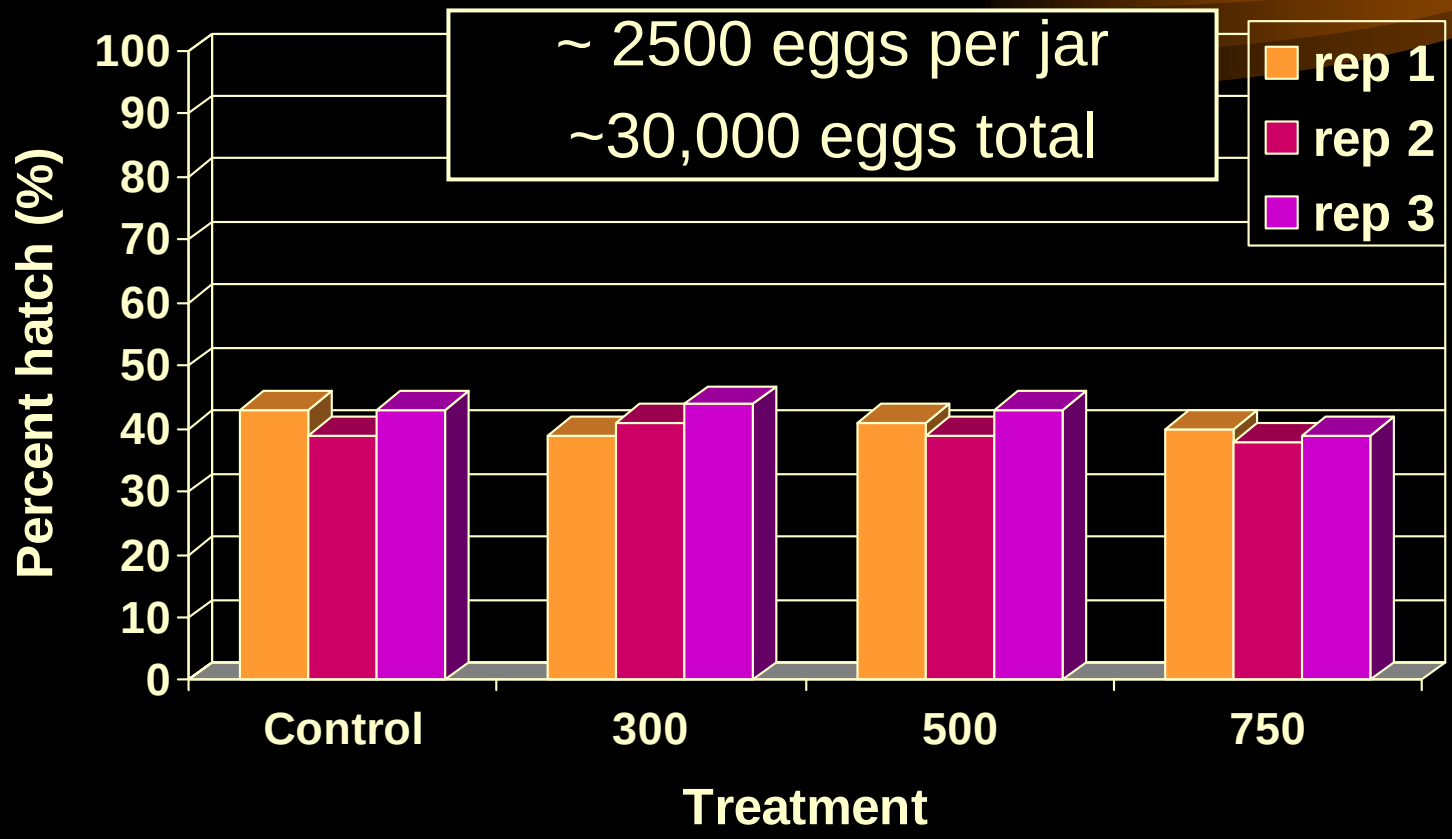
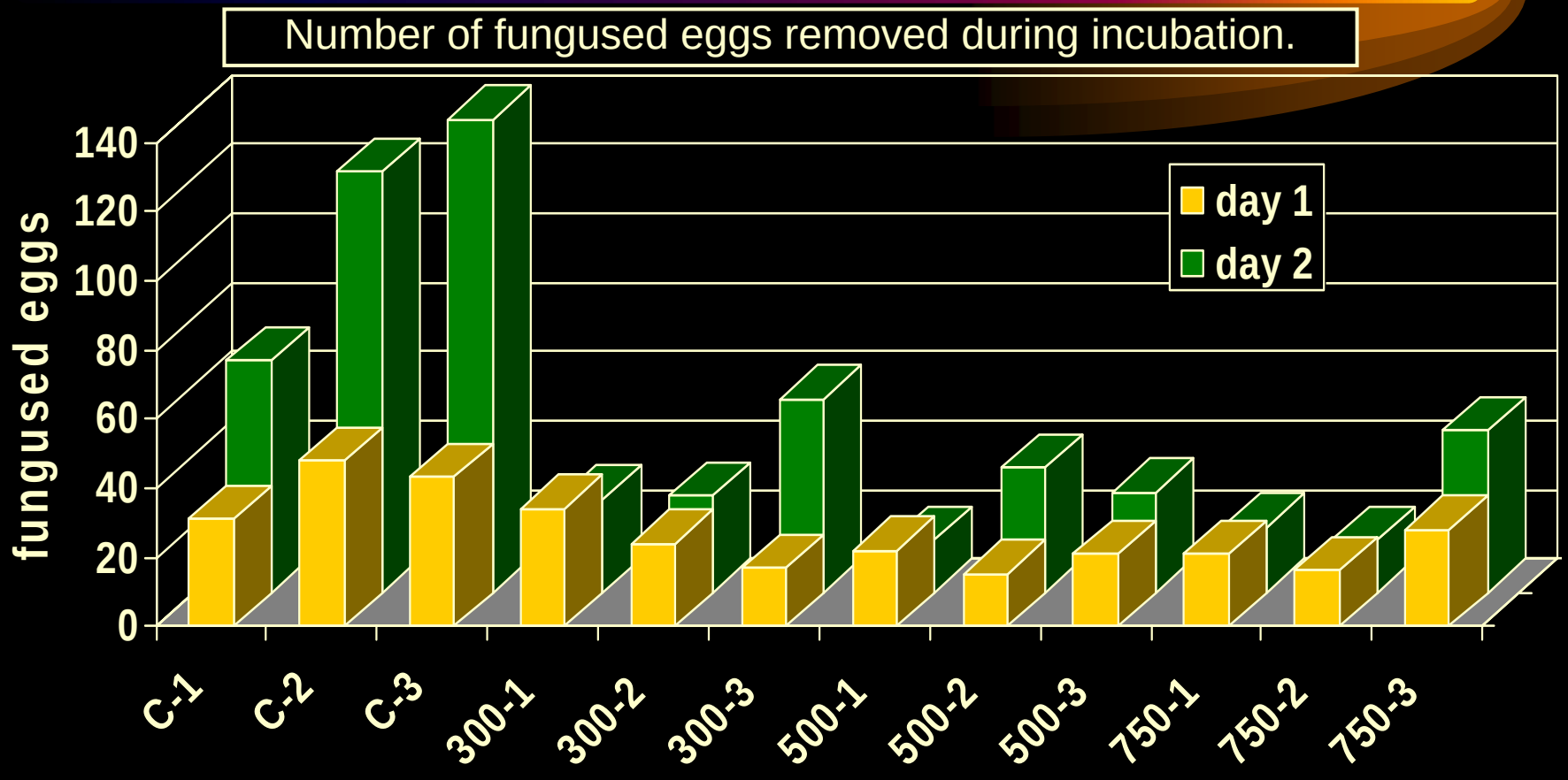
UMESC - Walleye



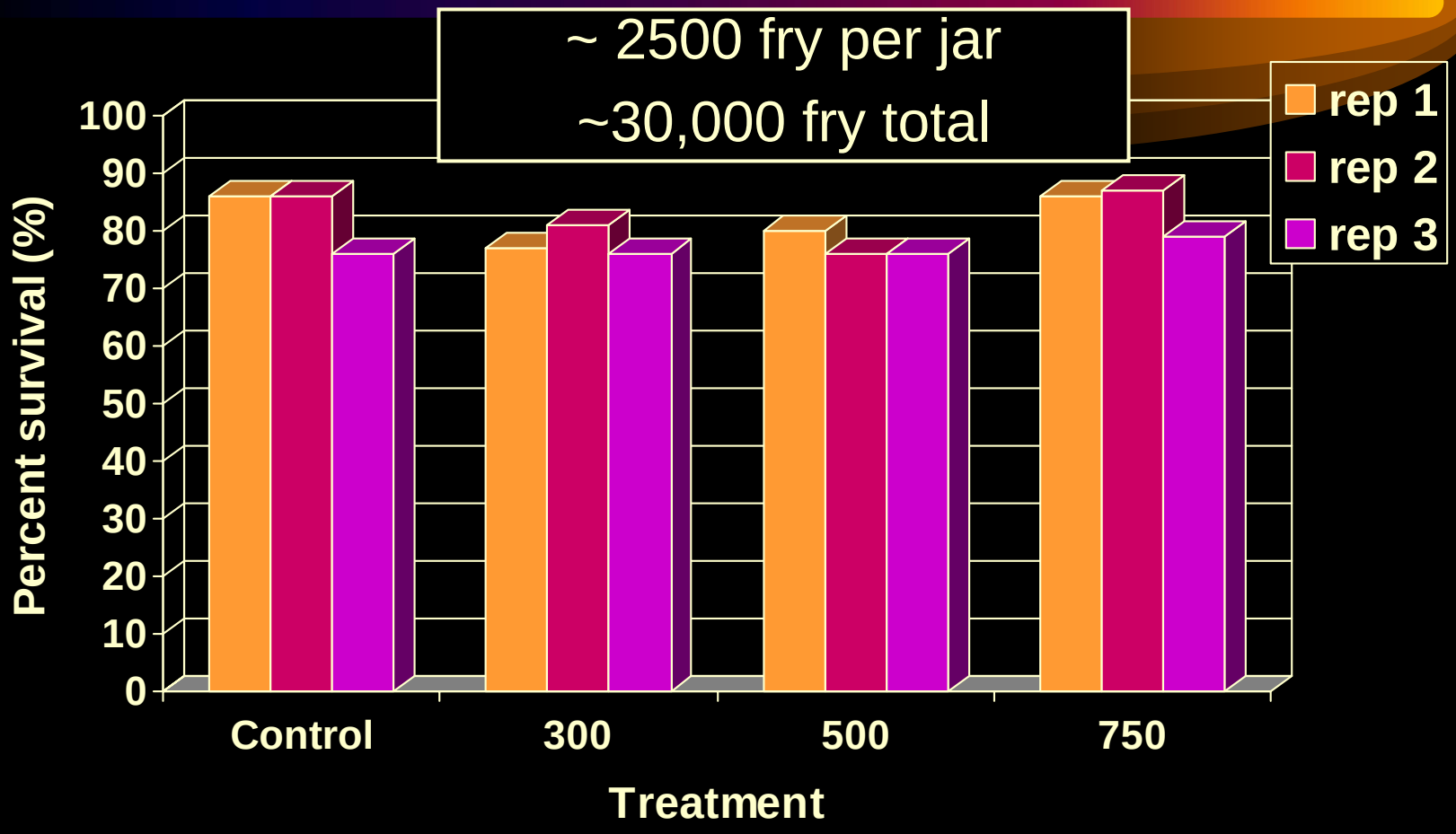
Dundee SFH - smallmouth bass eggs



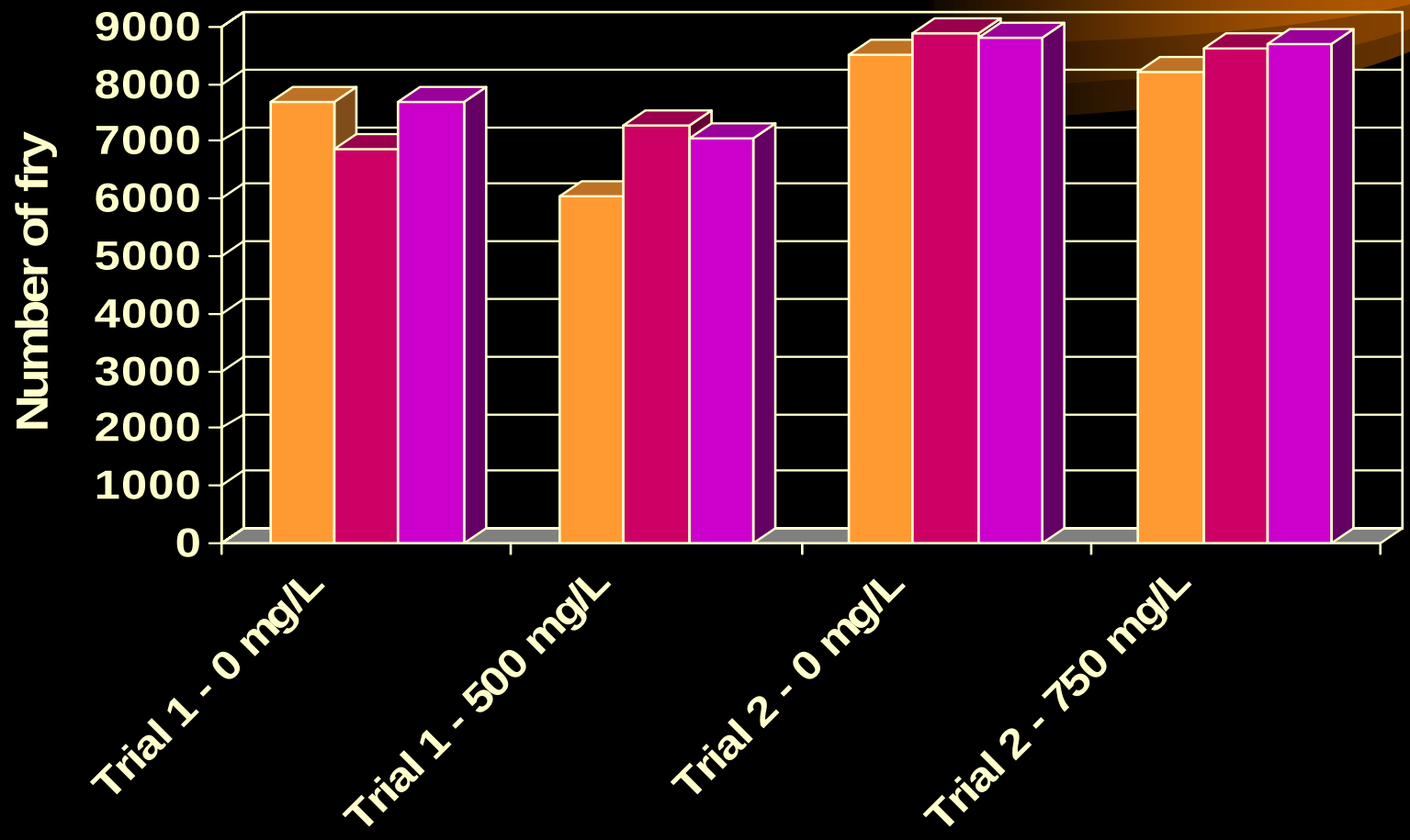
Dundee SFH - smallmouth bass eggs



Dundee SFH - smallmouth bass fry



Rathbun SFH - channel catfish eggs



Rathbun SFH - channel catfish control eggs

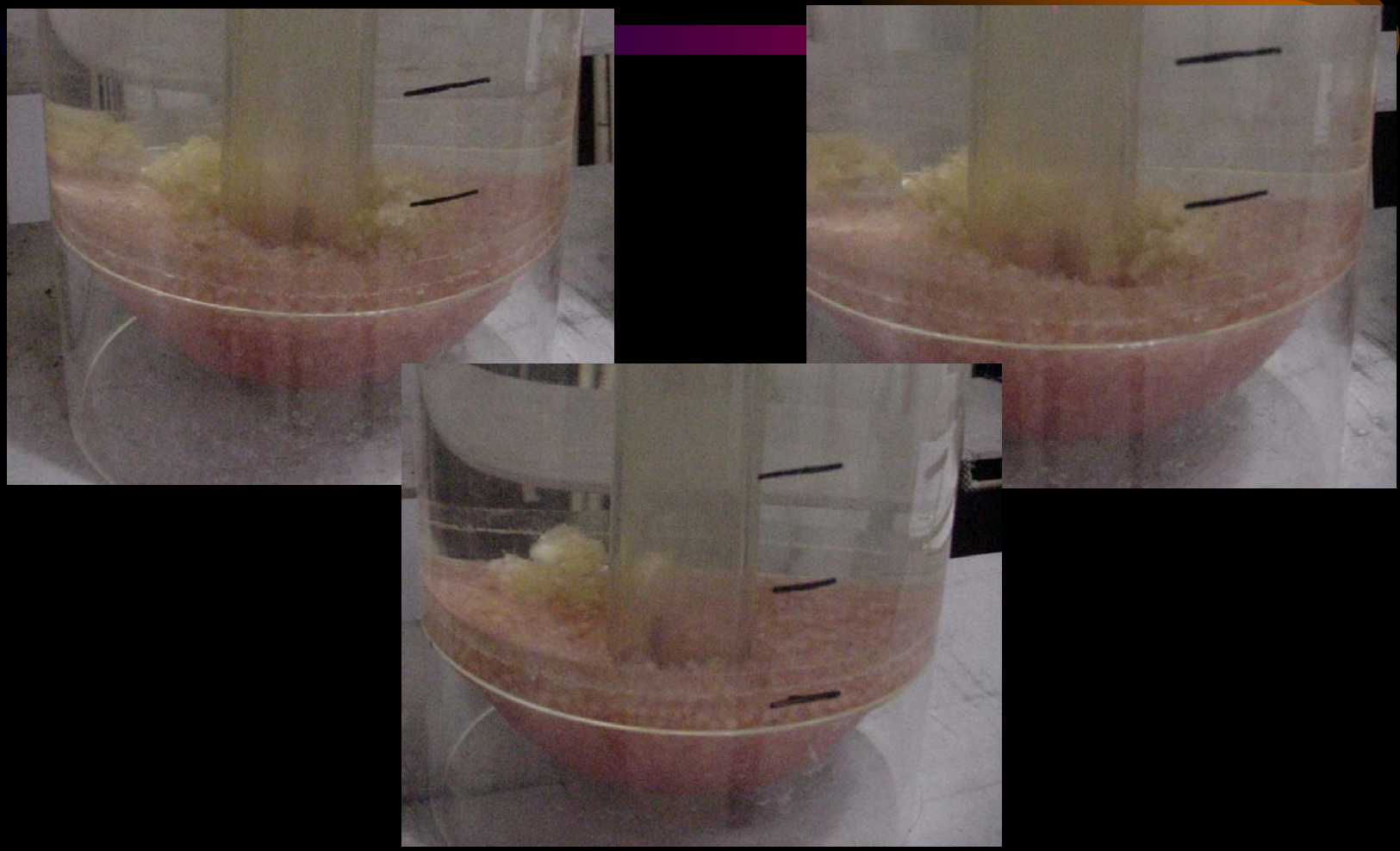


Photo credit: Andy Moore, Rathbun SFH

Rathbun SFH - channel catfish treated eggs

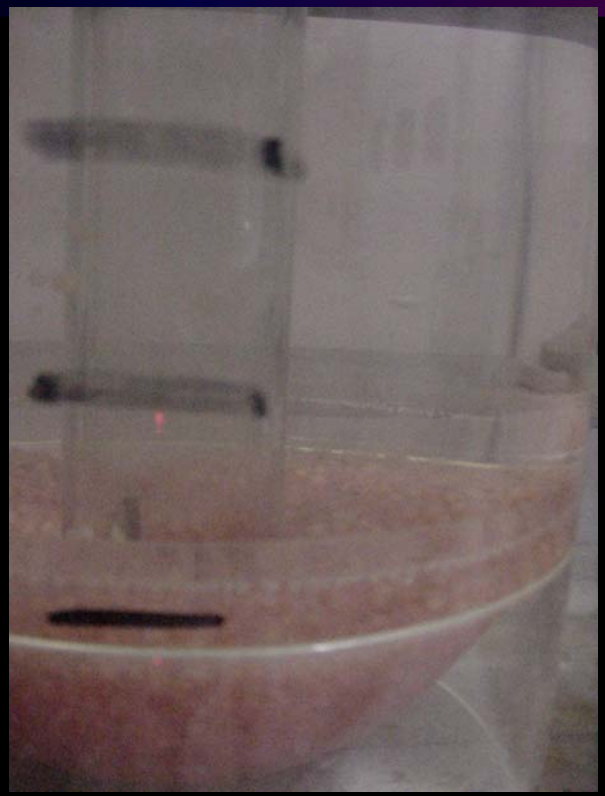


Photo credit: Andy Moore, Rathbun SFH

IAFWA Research Update

Aquaculture Therapeutant Environmental Assessment

How could we address the obvious data gaps when developing Environmental Safety submissions for aquaculture drugs?

Environmental Assessment Survey Goals

- Collect information directly from the hatchery - straight from the source
- Involve state, federal, and private facilities
- Develop data that could be used to provide background data for multiple drugs - both current and future

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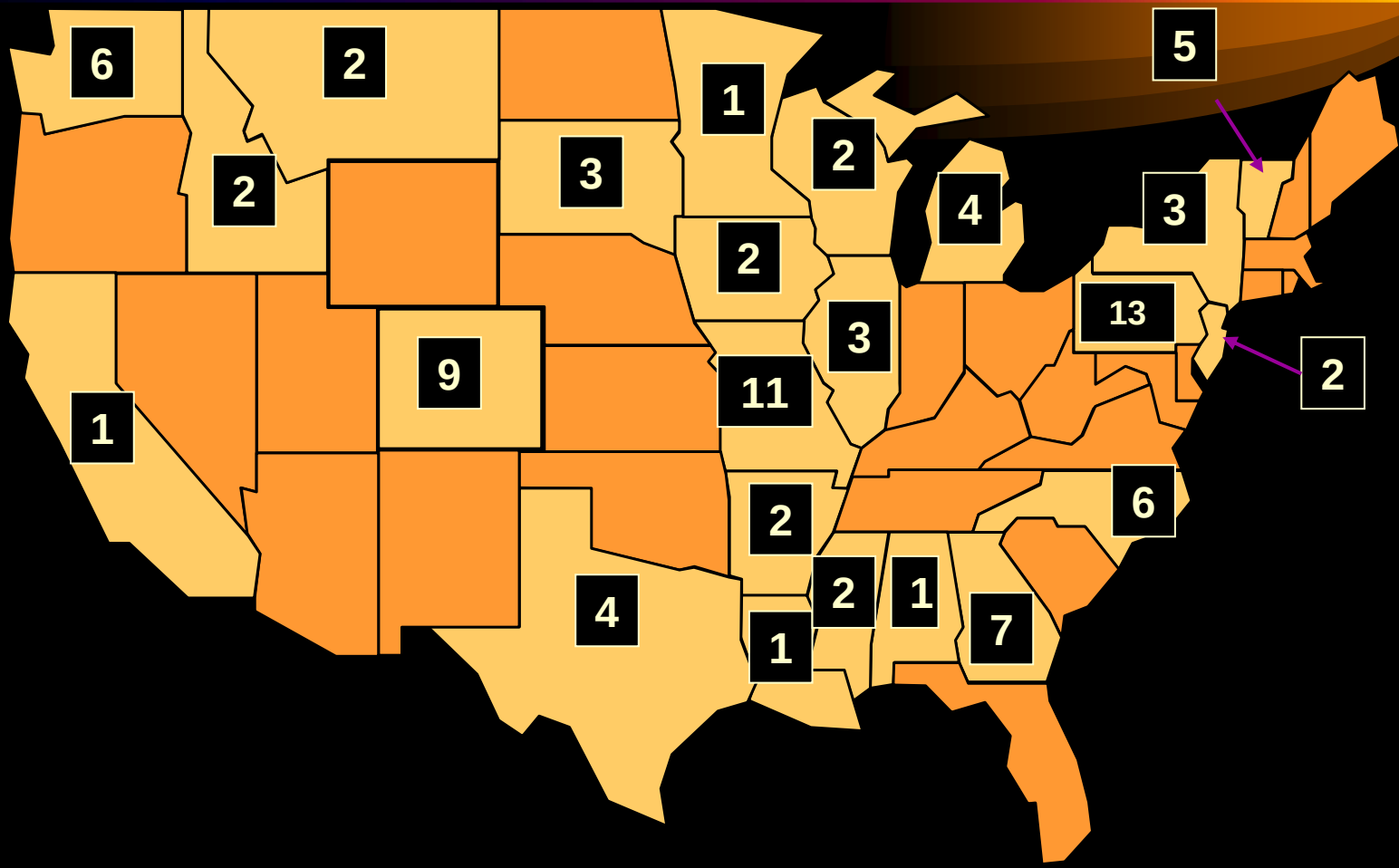
Who Responded?



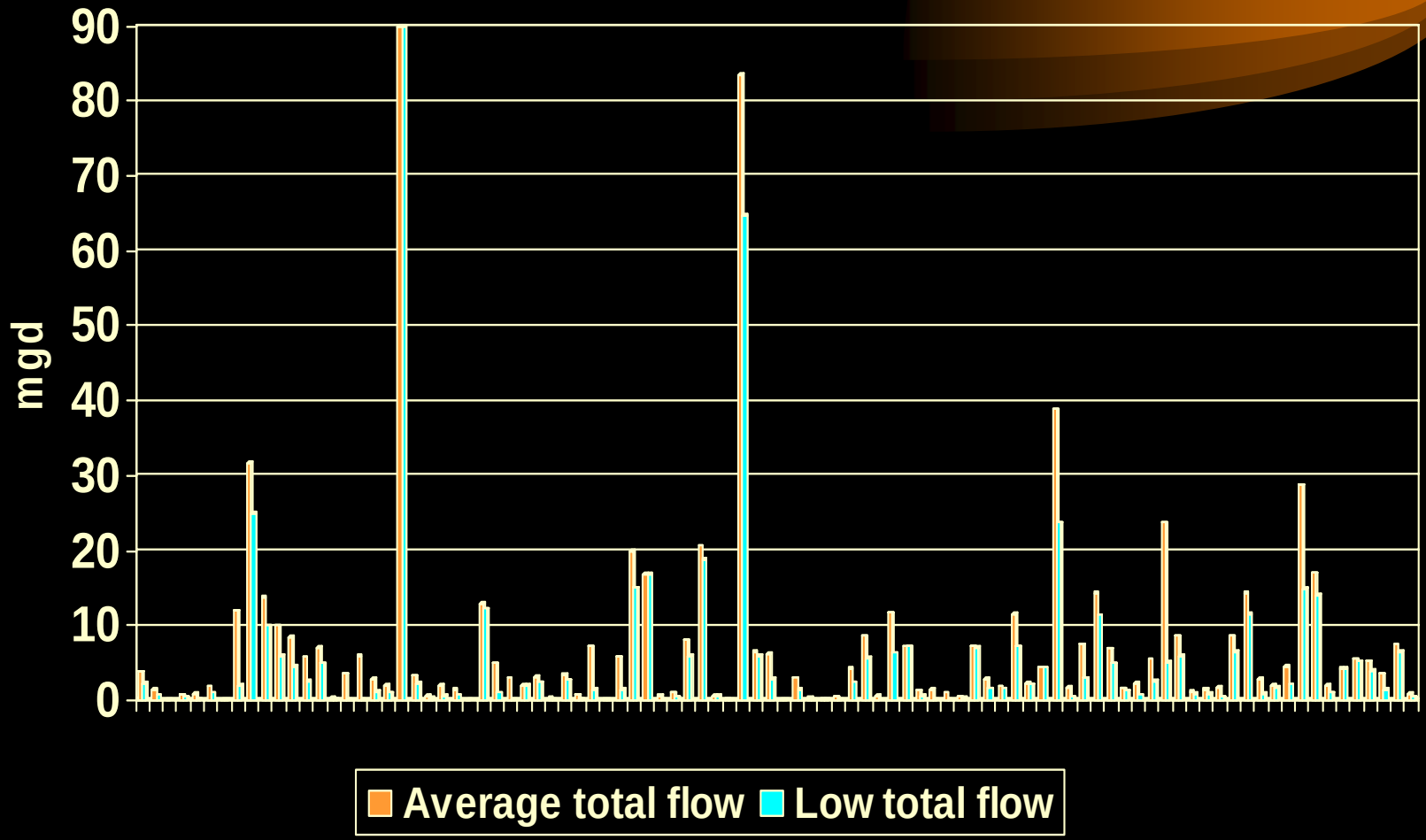
How were responses used?

- **Surveys were checked for accuracy and follow-up contacts made as needed**
- **Hydrogen peroxide EA**
  - **Data from 92 hatcheries**
  - **36 hatcheries would treat fish with H_2O_2**
  - **31 hatcheries would treat eggs with H_2O_2**

General Hatchery Discharge Characteristics

- 48 hatcheries discharged into settling ponds
  - median settling pond volume - 3.1 acre-feet
- 71 hatcheries discharged into a river or stream
  - median average flow - 26 cfs
- 21 discharged into lakes or backwaters
  - lake median volume - 11,250 acre-feet
  - backwater median volume - 55 acre-feet

Hatchery Water Use



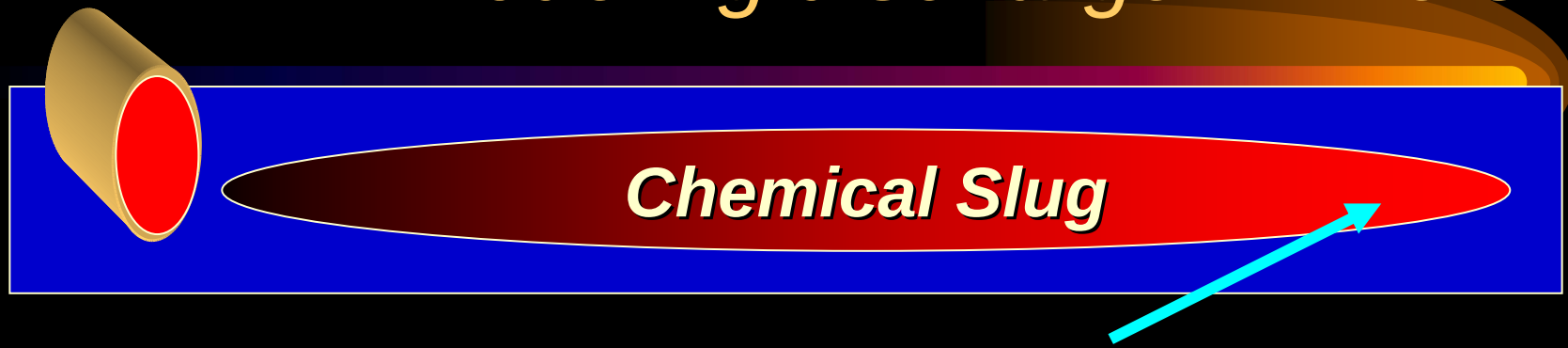
Estimating Environmental Concentrations

- Typical Treatment
  - maximum reported treatment concentration
  - maximum treatment volume
  - maximum treatment duration
  - maximum culture unit flow
  - average total hatchery flow
  - average receiving water flow/volume

Estimating Environmental Concentrations

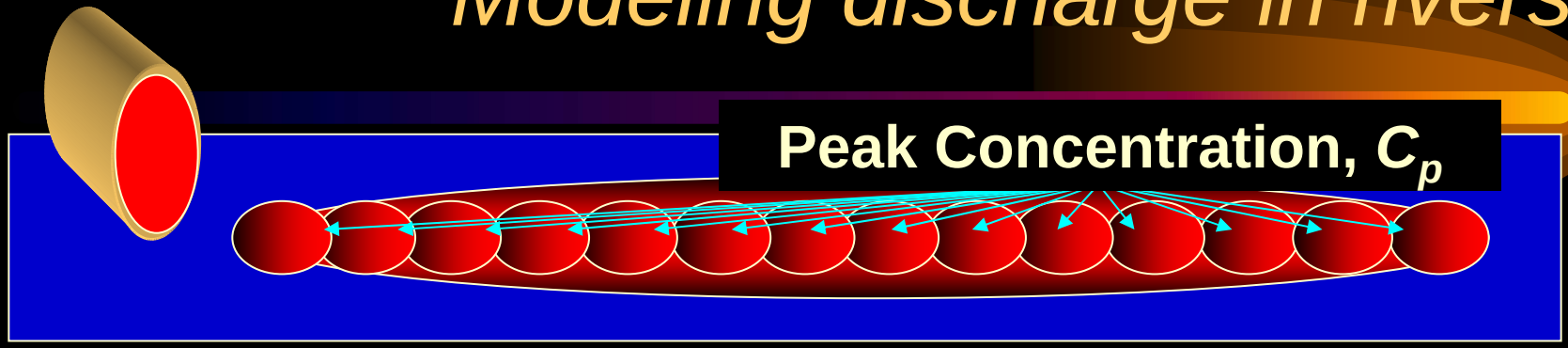
- Worst-Case Treatment
  - maximum ***LABELED*** concentration
  - maximum treatment volume
  - maximum treatment duration
  - maximum culture unit flow
  - ***LOW*** total hatchery flow
  - ***LOW*** receiving water flow/volume

Modeling discharge in rivers

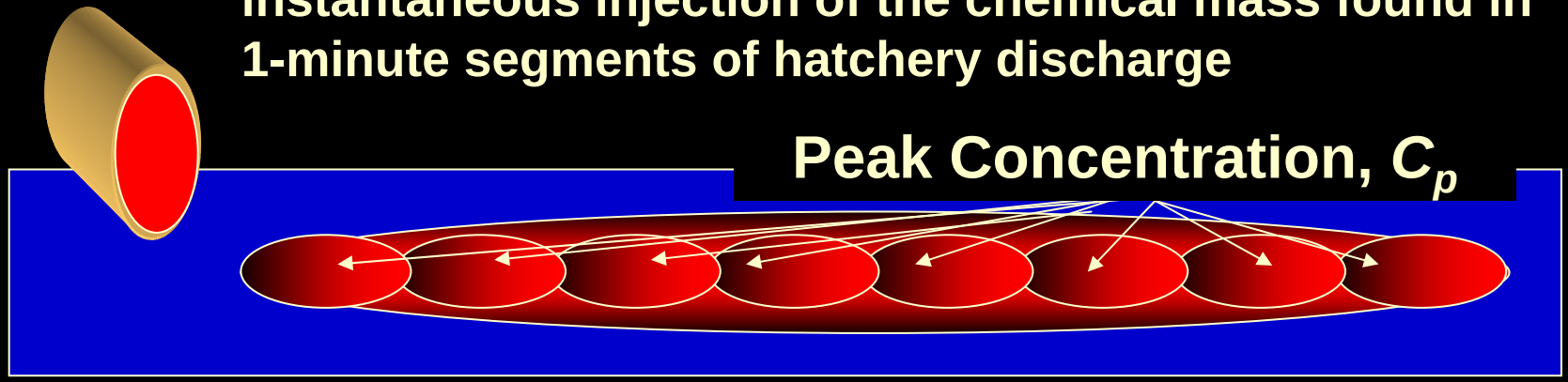


- Most chemical mixing models assume instantaneous discharge of the entire chemical mass in to a river or stream.
- Instantaneous discharge is unlikely to occur during or after chemical treatment at fish hatcheries.

Modeling discharge in rivers



Hatchery discharge model developed by using the instantaneous injection of the chemical mass found in 1-minute segments of hatchery discharge



Longitudinal dispersion of 1-minute segments of hatchery discharge at some time period and distance downstream from the discharge point.

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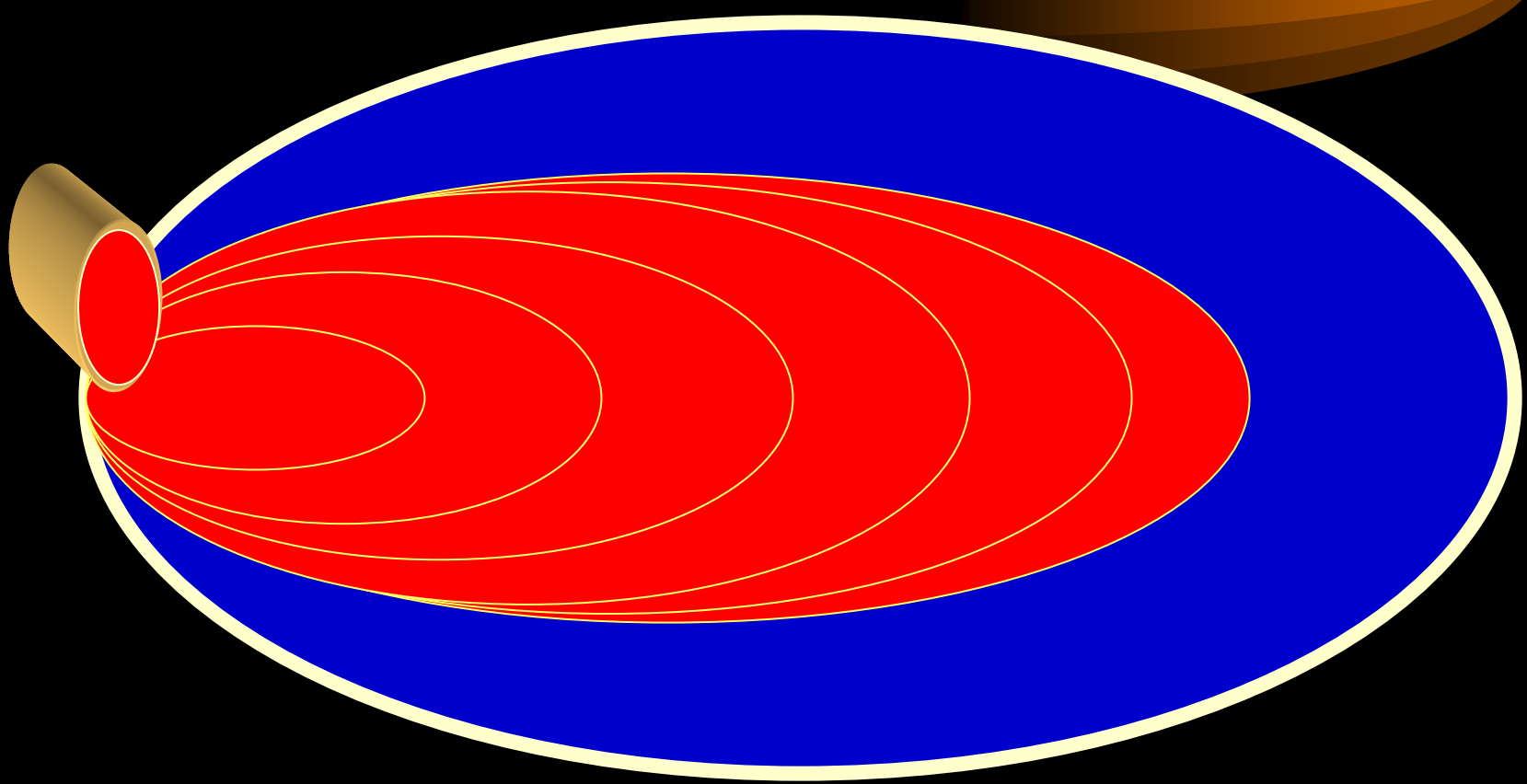
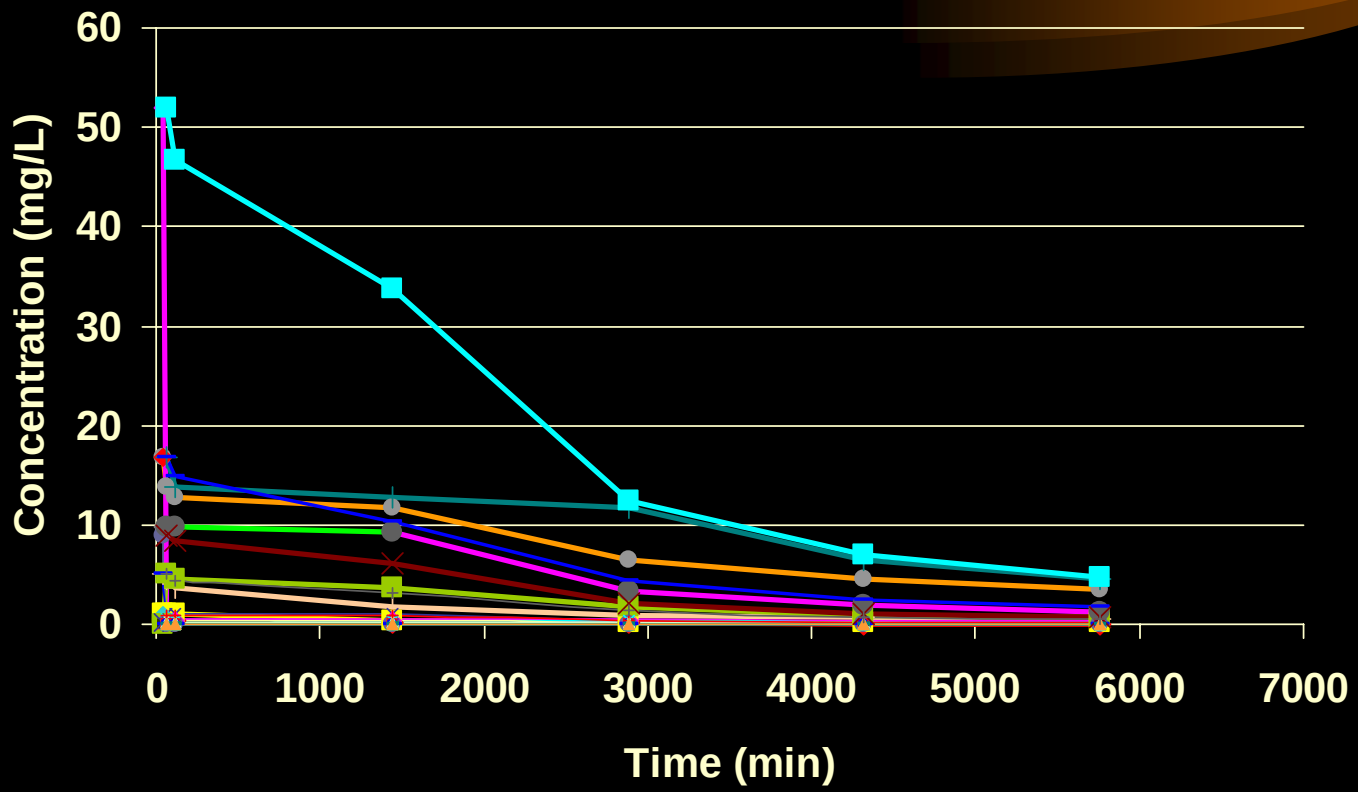
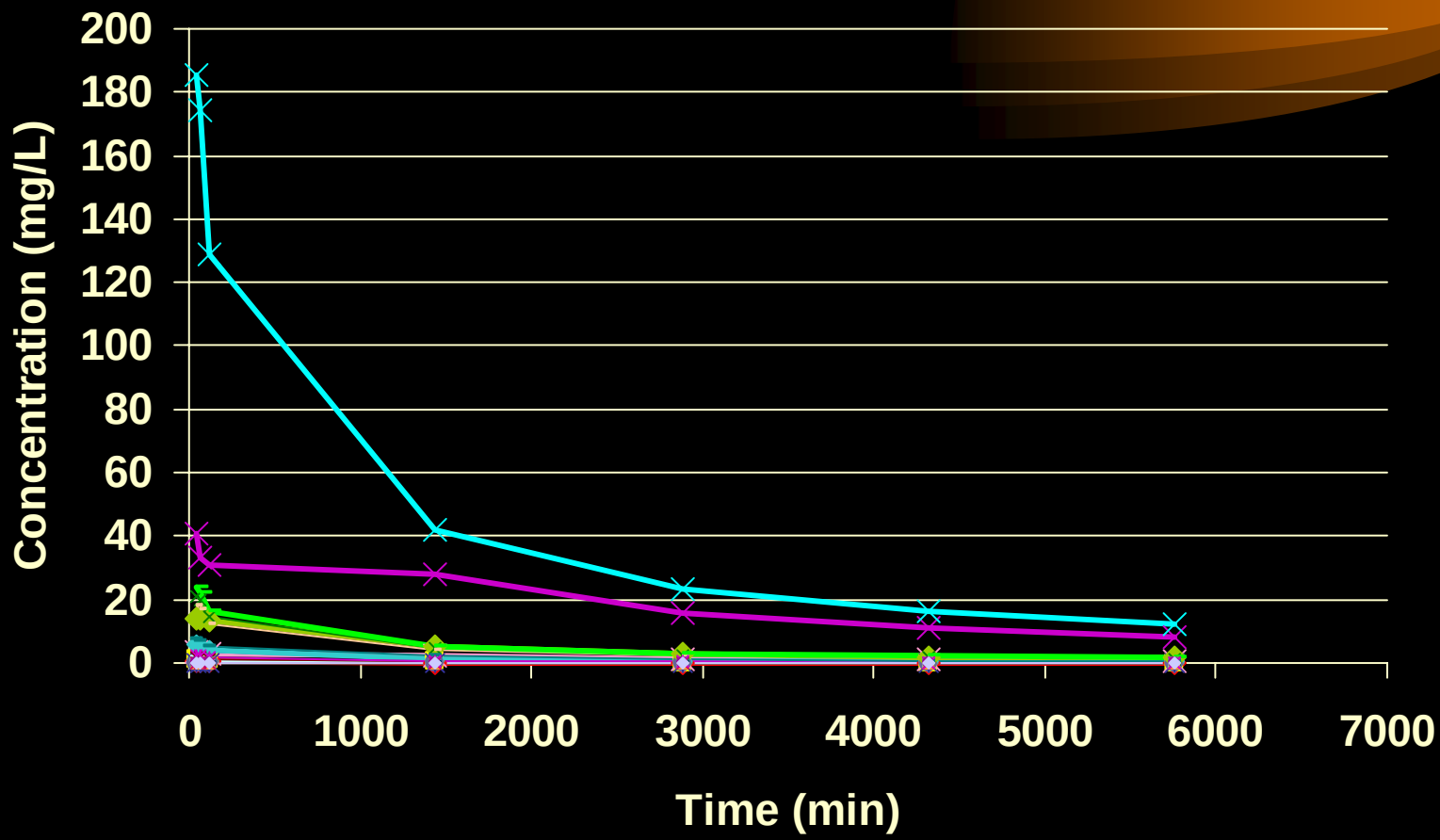
Modeling discharge into lakes and backwaters



Hydrogen peroxide EECs after a typical egg treatment

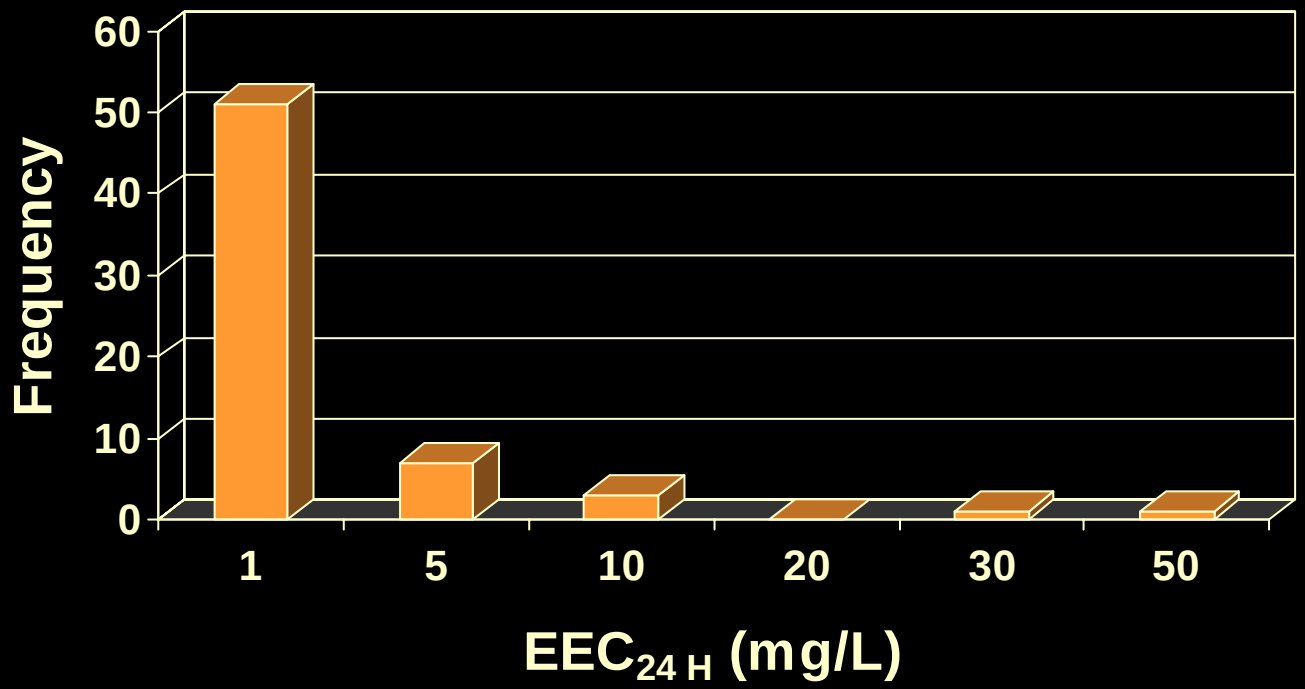


Hydrogen peroxide EECs after a worst-case egg treatment



Distribution of EECs 24 h after a "worst-case" treatment discharge

$EEC_{24\ H}$ (mg/L) resulting from worst-case hatchery discharge events



Risk Characterization

- Risk Ratios
  - Hazard Quotient and Risk Quotient
    - Provide an estimate of risk based on estimated environmental concentrations and laboratory toxicity information
- Risk ratios for hydrogen peroxide EECs based on survey data suggest no impact to the environment following treatment